REMARKS

Claims 1 to 7 and 9 to 73 are pending. Claims 1, 2, 4, 7, 9, 15 to 21, 29, 30, 40, 41, 47, and 48 stand allowed. Claims 3, 5, 6, 10 to 14, 22 to 27, 31 to 33, 35 to 39, 42 to 46, 49 to 55, and 59 to 73 stand rejected. Claims 28, 34, and 56 to 58 stand objected to. Applicants are herein amending claims 2, 4, 13, 14, 22, 23, 29, 50 to 52, 53, 54 to 61, 67, 69, 71 to 73 and canceling claims 1, 3, 10, 11, 62 to 65, without prejudice or disclaimer.

Amendments to Claims

Applicants are amending claims 2, 14, 23, 51, 53 to 61, 69, 67, 69, and 71 to 73 to correct errors as to form and/or claim dependency.

Applicants are amending claims 4, 22, 29, and 52 to specify that "Het" includes bithiophenyl. Support for the amendment may be found in original claim 2, wherein R^4 is "Het" where "Het" is bithiophenyl.

Applicants are amending claims 4, 22, and 52 to incorporate a proviso from claim 1 and claim 23 (and canceling claim 1 and amending claim 23 to delete the proviso as redundant).

Applicants are amending claims 22 and 52 to expand a proviso to include R^1 is "H" or NH_2". Support for this amendment to the proviso may be found in the proviso "if Z and Y are absent, R^4 is phenyl or 4-methoxyphenyl, R, R^1, R^2 and R^3 are H,...".

Applicants are amending claims 13, 50, and 60 to more clearly specify that the method of preventing adhesion of substances to a foreign substance involve the step of preparing a foreign surface where the foreign surface (such as an implant, catheter, or heart pacemaker) comprises the compounds of the invention. Support for this amendment may be found in original claim 9, which indicates the compounds of formula I may used "as anti-adhesive substances for implants, catheters or heart pacemakers."

Applicants are herein canceling claims 1, 3, 10, 11, 62 to 65, without prejudice or disclaimer. Applicants reserve the right to file one or more continuing applications to the cancelled subject matter.

Applicants submit that the amendments to the claims do not introduce new matter and are fully supported by the specification and claims1, as originally filed. Applicants respectfully request the Examiner to enter the amendments under 37 C.F.R. § 1.116(b) because the amendments either cancel claims or present the rejected claims in better form for consideration on appeal.

Rejection under 35 U.S.C. § 112, First Paragraph (Non-enablement)

Claims 5, 6, 12, 31, 32, 35, 38, 39, 42, 45, 46, 49, 55, 56, and 59 are rejected under 35 U.S.C. § 112, first paragraph, because the specification allegedly does not enable the treatment of certain sequelae of thrombotic disorders, namely myocardial infarct, arteriosclerosis, angina pectoris, acute coronary syndrome, stroke, transient ischemic attack, or reocclusion/restenosis after angioplasty/stent implantation. The Office Action acknowledges that the specification is enabling for the treatment of thrombosis and peripheral circulatory disorders. Applicants traverse the rejection because the specification provides ample information for the skilled artisan to make and use the claimed methods of treatment without undue experimentation.

The crux of the rejection in the Office Action appears to be that the applicants have allegedly not shown the correlation between antagonizing glycoprotein IbIX and the treatment of certain sequelae of thrombotic disorders, namely myocardial infarct, arteriosclerosis, angina pectoris, acute coronary syndrome, stroke, transient ischemic attack, or reocclusion/restenosis after angioplasty/stent implantation. It is further reasoned in the Office Action that while there is a correlation between antagonizing glycoprotein IbIX and thrombosis, there seems to be no correlation between thrombosis and the above-listed sequelae and hence no correlation between antagonizing glycoprotein IbIX and the above-

listed sequelae. Applicants respectfully traverse these conclusions, as the specification explains the art-recognized link between the antagonism of glycoprotein IbIX and thrombosis as well as the further link between antagonism of glycoprotein IbIX and the sequelae of thrombotic disorders, as explained more fully below.

Applicants explain in the specification on page 3, line 1 to page 4, line 19 that the compounds of the quinazolinone compounds of the invention are glycoprotein IbIX inhibitors and how such inhibitors play a role in thrombus formation and they may be used to prevent and/or treat thrombotic disorders and sequelae deriving therefrom:

> They act especially as GPIbIX inhibitors, in particular inhibiting the interaction of this receptor with the ligand von Willebrand factor (vWF). This action can be demonstrated, for example, by a method which is described by S. Meyer *et al.* in *J. Biol. Chem.* 1993, 268, 20555-20562. The property as GPIbIX alpha-thrombin receptor (N. J. Greco, *Biochemistry* 1996, 35, 915-921) can also be blocked by the compounds mentioned.
>
> The significance of GPIbIX as an adhesion receptor on platelets, which mediates the primary interaction of platelets with an arteriosclerotically modified vascular wall via binding to the vWF expressed there, has been described by many authors (*e.g.* Z.M. Ruggeri in *Thromb. Hemost.* 1997, 78, 611-616). The activation of another platelet adhesion receptor, GPIIbIIIa, following the GPIbIX-vWF interaction, leads to platelet aggregation and thus to thrombotic vascular occlusion.
>
> A GPIbIX antagonist can thus prevent the start of thrombus formation and thus also release of active substances from the platelets which, for example, promote thrombus growth and have an additional trophic action on the vascular wall. This has been shown with inhibitory peptides or antibodies in various experimental models (*e.g.*, H Yamamoto *et al., Thromb. Hemost.* 1998, 79, 202-210).
>
> In the case of higher shear forces, the blocking action of GPIbIX inhibitors exerts its maximum effect, as described by J. J. Sixma *et al.* in *Arteriosclerosis, Thrombosis, and Vascular Biology* 1996, 16, 64 71. According to the flow chamber method used there, the compounds of the formula I can be characterized as GPIbIX inhibitors in whole blood.
>
> The inhibition of thrombus formation of the GPIbIX inhibitors can be measured by a modified Born method (*Nature* 1962, 4832, 927-929) using botrocetin or ristocetin as an aggregation stimulant.

> The compounds of the formula I according to the invention can therefore be employed as pharmaceutical active compounds in human and veterinary medicine. They act as adhesion receptor antagonists, in particular as glycoprotein IbIX antagonists, and are suitable for the prophylaxis and/or therapy of thrombotic disorders and sequelae deriving therefrom. The preferentially best action is to be expected in the case of thrombotic disorders in the arterial vascular system, but GPIbIX inhibitors also have an effect in the case of thrombotic disorders in the venous vascular bed. The disorders are acute coronary syndromes, angina pectoris, myocardial infarct, peripheral circulatory disorders, stroke, transient ischaemic attacks, arteriosclerosis, reocclusion/restenosis after angioplasty/stent implantation. The compounds can furthermore be employed as anti-adhesive substances where the body comes into contact with foreign surfaces such as implants, catheters or cardiac pacemakers.

In order to establish a *prima facie* case of non-enablement, the following must be established by the Patent Office:

1. a rational basis as to
   a. why the disclosure does not teach; or
   b. why to doubt the objective truth of the statements in the disclosure that purport to teach;
2. the manner and process of making and using the invention
3. that correspond in scope to the claimed invention
4. to one of ordinary skill in the pertinent technology,
5. without undue experimentation, and
6. dealing with subject matter that would not already be known to the skilled person as of the filing date of the application.

Any rejection under 35 U.S.C. § 112, second paragraph, for lack of enablement, must include evidence supporting each of these elements. Applicant respectfully submits that the Office has failed to meet its burden of establishing a *prima facie* case of non-enablement.

It has been consistently held that the first paragraph of 35 U.S.C. § 112 requires nothing more than *objective* enablement. Furthermore, a specification that teaches how to make and use the invention in terms which correspond in scope to the claims *must* be taken as

complying with the firs paragraph of 35 U.S.C. § 112, *unless* there is reason to doubt the objective truth of the statements relied upon therein for enabling support. *Stahelin v. Secher*, 24 U.S.P.Q.2d 1513, 1516 (B.P.A.I. 1992) (citing *In re Marzocchi*, 439 F.2d 220, 169 USPQ 367 (C.C.P.A. 1971). "[I]t is incumbent upon the Patent Office, whenever a rejection on this basis is made, to ... back up assertions of its own with acceptable evidence or reasoning which is inconsistent with the contested statement." *In re Marzocchi*, 439 F.2d 220, 224, 169 U.S.P.Q. 367, 370 (C.C.P.A. 1971). In the instant application, no evidence has been provided of why the disclosure is insufficient or why the Office does not believe the statements contained therein. Accordingly, applicant respectfully submits that the Office has not met its burden.

As described above, applicants have provided a link between the antagonism of glycoprotein IbIX and thrombosis and the sequelae therefrom. In addition, the specification provides detailed instruction for the synthesis of 29 compounds (page 37, line 21 to page 90, line 2) and eight types of pharmaceutical dosage forms (page 91, line 5 to page 92, line 20). Furthermore, the specification provides general guidelines for formulating the compounds with pharmaceutical carriers (page 35, line 26 to page 36, line 13) and dosage levels of the compounds for use in methods of treating thrombotic disorders and sequelae deriving therefrom (page 36, line 15 to page 37, line 3).

No reasonable evidence has been provided that contradicts that the compounds of the invention or any glycoprotein IbIX antagonists could be used for the prevention and/or treatment of certain sequelae of thrombotic disorders. Applicants are enclosing a number of further references, beyond those discussed in the specification, which show that the correlation between thrombosis and the sequelae deriving therefrom:

(1) Sigma RBI, sigma-aldrich.com/cellsignaling
(thrombus formation -- acute myocardial infarction and stroke)

(2) Charakida, *et al., Hellenic J. Cardiol.* 44: 43-48, 2003
(thrombotic disposition -- risk of acute coronary syndromes, atherosclerotic disease)

(3) The Merck Manual, Section 11, Chapter 132

(thrombotic disorders -- atherosclerosis, stroke, myocardial infarct, and peripheral artery occlusion)

(4) www.hemosense.com
(thrombotic disorders – myocardial infarction, angina, acute ischemic stroke)

(5) Gils, *et al., Critical Care Nurse,* April 2000, Volume 20, Number 2
(thrombus formation – acute coronary syndrome, unstable angina, myocardial infarction, atherosclerotic plaque)

(6) US-B-6,280,731
(antithrombotic agent – stroke, myocardial infarction, angina pectoris, peripheral arterial occlusive disease, prevention of reocclusion after PTCA and occlusion of coronary artery by-pass graft)

(7) US-A-5,858,972
(antithrombotic agent – myocardial infarction, angina pectoris, stroke)

A lack of working examples with respect to *in vitro* or *in vivo* assays does not automatically make a patent non-enabling. *Atlas Powder Co. v. E.I. du Pont de Nemours & Co.*, 224 U.S.P.Q. 409 (Fed. Cir. 1984). Additionally, 35 U.S.C. § 112 does not demand a "working example," and an application cannot be fatally defective merely because it lacks one. *In re Long*, 151 U.S.P.Q. 640 (C.C.P.A. 1966); *In re Honn et al.*, 150 U.S.P.Q. 652 (C.C.P.A. 1966); *In re Bartholome et al.*, 156 U.S.P.Q. 20 (C.C.P.A. 1967); and *Ex parte Kenega*, 189 U.S.P.Q. 62 (Pat. Off. Bd. App. 1974).

Because the non-enablement rejection is not supported by sufficient evidence that the methods employing the compounds of the invention cannot be made and used in the manner described in the specification without undue experimentation, applicants respectfully submit that there is not a reasonable basis for rejecting the claims. Accordingly, applicants respectfully request reconsideration and withdrawal of the rejection of the claims 5, 6, 12, 31, 32, 35, 38, 39, 42, 45, 46, 49, 55, 56, and 59 under 35 U.S.C. § 112, first paragraph, for being non-enabling.

Rejection under 35 U.S.C. § 112, First Paragraph (Lack of Written Description)

Claims 13, 14, 36, 37, 43, 44, 50, 51, 60, 61, 66 to 73 are rejected under 35 U.S.C. § 112, first paragraph, because the claims allegedly lack adequate written description with respect to the use of the compounds as anti-adhesion agents for foreign surfaces. Applicants are herein amending claims 13, 50, and 60 to more clearly specify that the method of preventing adhesion of substances to a foreign substance involves the step of preparing a foreign surface where adhesion is presented, where the foreign surface (such as an implant, catheter, or heart pacemaker) *comprises* the compounds of the invention. Support for this amendment may be found in original claim 9, which states the compounds of formula I may used "as anti-adhesive substances for implants, catheters or heart pacemakers." Applicants submit that the specification and claims, as filed, clearly show that the applicants were in possession of the invention claimed in claims 13, 14, 36, 37, 43, 44, 50, 51, 60, 61, 66 to 73, and applicants have further amended the claims to clarify this possession.

Accordingly, applicants submit that they have met the written description requirements and request withdrawal of the rejection of claims 13, 14, 36, 37, 43, 44, 50, 51, 60, 61, 66 to 73 under 35 U.S.C. § 112, first paragraph, for lacking written description.

Rejection under 35 U.S.C. § 112, Second Paragraph

Claims 3, 10, 11, and 62 to 65 are rejected under 35 U.S.C. § 112, second paragraph. Applicants are herein canceling claims 3, 10, 11, and 62 to 65, without prejudice or disclaimer, thereby rendering moot the rejection. Accordingly, applicants request withdrawal of the rejection of claims 3, 10, 11, and 62 to 65 under 35 U.S.C. § 112, second paragraph.

Rejection under 35 U.S.C. § 102(b)

Claims 22, 24 to 27, 33, 52, and 53 are rejected under 35 U.S.C. § 102(b), as more specifically set forth below:

Claims 22, 24 to 26, and 33 are rejected under 35 U.S.C. § 102(b) as allegedly anticipated by Bhaduri, *et al.* Applicants are herein amending claim 22 to modify the first proviso such that R^1 is H or NH_2. As amended, this proviso excludes the compounds disclosed by Bhaduri, *et al.* Accordingly, applicants request withdrawal of the novelty rejection with respect to Bhaduri, *et al.*

Claims 22, 24 to 27, 52, and 53 are rejected under 35 U.S.C. § 102(b) as allegedly anticipated by Dean, *et al.* Applicants are herein amending claims 4, 22, and 52 to incorporate the proviso:

> if Z and Y are absent, R^4 is phenyl or 4-methoxyphenyl, R, R^1, R^2 and R^3 are H, then the sum of n and m is not 2 or 3

With this proviso, claims 4, 22, and 52 exclude the compounds disclosed by Dean, *et al.* Accordingly, applicants request withdrawal of the novelty rejection with respect to Dean, *et al.*

Claims 22, 24 to 27, and 52 are rejected under 35 U.S.C. § 102(b) as allegedly anticipated by Nielsen, *et al.* Applicants are herein amending claims 22 and 52 to modify the proviso

> if Z is absent, Y is absent or vinyl, R^4 is phenyl, phenylalkyl, alkoxyphenyl or pyridyl, R is H and R^1 is H or NH_2, then R^2 and R^3 are not A,

such that R^1 is H or NH_2. As amended, this proviso excludes the compounds disclosed by Nielsen, *et al.* Accordingly, applicants request withdrawal of the novelty rejection with respect to Nielsen, *et al.*

Claims 22 to 27, and 52 to 55 are rejected under 35 U.S.C. § 102(b) as allegedly anticipated by Zimaity, *et al.* Applicants are herein amending claims 22 and 52 to modify the proviso

if Z is absent, Y is absent or vinyl, R^4 is phenyl, phenylalkyl, alkoxyphenyl or pyridyl, R is H and R^1 is H or NH_2, then R^2 and R^3 are not A,

such that R^1 is H or NH_2. As amended, this proviso excludes the compounds disclosed by Zimaity, *et al.* Accordingly, applicants request withdrawal of the novelty rejection with respect to Zimaity, *et al.*

Applicants respectfully submit that claims 4, 22, 24 to 27, 33, 52, and 53 are not anticipated by Bhaduri, *et al.*, Dean, *et al.*, Nielsen, *et al.*, or Zimaity, *et al.* Accordingly, applicants request withdrawal of the rejection of claims 22, 24 to 27, 33, 52, and 53 under 35 U.S.C. § 102(b).

Claim Objections

Claims 28, 34, and 56 to 58 are objected to as dependent from a rejected claims, but are deemed otherwise allowable. Applicants are herein amending claims 22 and 52, the claims from which claims 28, 34, and 56 to 58 depend directly or indirectly. In view of these amendments, applicants submit that claims 28, 34, and 56 to 58 are patentable. Accordingly, applicants request withdrawal of the objection to claims 28, 34, and 56 to 58.

Conclusions

Applicants request:

(1) entry of the amendment;

(2) reconsideration and withdrawal of the objection and rejection of the claims; and

(3) allowance of claims 2, 4 to 7 and 9, 12 to 61, and 66 to 73.

If the Examiner is of a contrary view, the Examiner is requested to contact the undersigned attorney at (215) 557-3861.

Date: June 17, 2004

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Protein Tyrosine Phosphatases...(continued)

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New Product Highlights

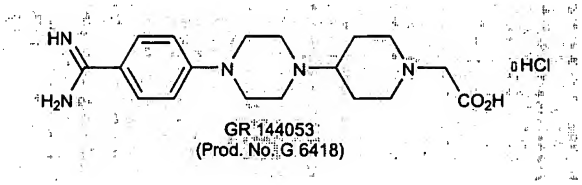
GR 144053: Non-peptide antagonist of the platelet glycoprotein IIb/IIIa (GP IIb/IIIa) fibrinogen receptor

Following vascular injury, platelets become activated and adhere to damaged blood vessel walls and exposed sub-endothelial connective tissues thereby forming the initial platelet plug. Platelets play a central role in thrombus formation and are known to participate in many life-threatening thrombotic disorders such as acute myocardial infarction, stroke and pulmonary embolism. One platelet receptor involved in this activation is glycoprotein Ib/IX (GP Ib/IX) together with its ligand von Willebrand factor (vWF). Activation causes the exposure of phospholipids (PL) and the membrane glycoprotein IIb/IIIa (GP IIb/IIIa) on the platelet surface, providing a platform upon which the members of the coagulation cascade can assemble.

GR 144053 (Prod. No. **G 6418**) is a potent and selective, non-peptide antagonist at the glycoprotein IIb/IIIa (GP IIb/IIIa) fibrinogen receptor [1,2]. It acts as a mimetic of the peptide RGD-sequence, a potent inhibitor of GPIIb/IIIa. Binding of GR 144053 to GPIIb/IIIa competitively blocks the binding of its normal ligand, **fibrinogen** (Prod. No. **F 4883**), and alters the signaling properties of the GPIIb/IIIa heterodimer. It attenuates platelet aggregation, activation and degranulation both *in vivo* and *in vitro* and inhibits ADP-induced platelet aggregation with an IC_{50} value of 17.7 nM [2].

GR 144053 also suppresses the activation of platelets by **aurintricarboxylic acid** (ATA, Prod. No. **A 0885**) [2]. The molecular mechanism of ATA action has not been completely elucidated. One possible mechanism is through its binding to GP Ib, thereby blocking binding of vWF. This observation suggests additional activities for GR 144053 that are not mediated by the GP IIb-IIIa receptor [2].

GR 144053 is a useful tool for studying the of mechanisms of platelet activation and degranulation events. Currently, anti-thrombotic therapy includes anti-platelet, anti-coagulant, pro-thrombolytic or pro-fibrinolytic agents. GR 144053 may be potentially useful in achieving anti-thrombosis effects while maintaining the integrity of the vascular system.



GR 144053
(Prod. No. G 6418)

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Reviews

The Role of Platelet Glycoprotein Ib and IIb Polymorphism in Coronary Artery Disease

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In Western society atherosclerotic disease is one of the commonest causes of increased morbidity and mortality[1,2]. Atherosclerosis is a multifactorial disease and many different environmental factors such as physical inactivity, cigarette smoking, hormonal and genetical or acquired (inherited dyslipidaemia, hypertension, diabetes, positive family history of cardiovascular disease[3,4]) combine in order to determine its onset and outcome.

Especially within the last decade, several genes and their polymorphisms involved in the atherosclerotic process, have been found to increase thrombotic predisposition and risk of acute coronary syndromes. Among these genes, platelet glycoprotein polymorphisms have been studied intensely.

This article is a review regarding the role of two platelet glycoprotein polymorphisms Ib and IIb in cardiovascular thrombosis. It is also worth mentioning the difference between polymorphism and mutation. Mutation is defined any change (heritable or not) in DNA sequence while polymorphism is the difference in DNA sequence among individuals. The term polymorphism describes genetic variations occurring in more than 1% of the population.

Platelet receptors and their role in thrombosis

Most platelet receptors are protein complexes with two or more polypeptide subunits non-covalently associated with the platelet membrane[5]. In all stages of platelet adhesion and aggregation, these receptors interfere with subendothelial matrix. Following vascular injury and under high shear stress conditions[6], platelets adhere to the surface bound von Willebrand factor (vWF) through the platelet glycoprotein (GP) Ib/X/V. This adhesion is made more stable and secure by subsequent multiple interactions between glycoprotein Ia/IIa with collagen and GPIIb/IIIa and Ic/IIa with vWF and fibronectin respectively[6].

Given the importance of platelet glycoproteins in primary haemostasis, it is reasonable to suggest that in certain circumstances, inherited differences in these platelet receptors may contribute, by altering their activity, to an increased risk of acute coronary events. A platelet polymorphism, for instance, in a regulatory gene region may alter the expression of the receptor on the platelet surface. Moreover a nucleotide polymorphism that results in an amino acid substitution may change the tertiary structure of the receptor and subsequently change platelet adhesive function.

Glycoprotein GPIb/IX/V- Structure and polymorphism

This receptor consists of four subunits (proteins: GPIba, GPIbβ, GPIX and GPV)

that are the products of distinct genes[7]. These subunits have similar structural features and belong to the "leucine-rich family" of glycoproteins[8]. There are approximately 25.000 copies of this receptor per platelet[9,10]. Glycoprotein Ib is composed of two disulfide- linked polypeptides, glycoprotein Iba and glycoprotein Ibβ and this complex is non-covalently associated with glycoproteins GPIX and GPV[8,11,12]. The GPIb/IX/V receptor mediates the initial adhesion of platelets to the extracellular matrix under conditions of high shear stress via the binding of von Willebrand factor (vWf) to the amino acid terminal domain of glycoprotein Iba[13]. Given the importance of the GPIb/IX/V receptor in platelet adhesion it is reasonable to suggest that small alterations in GPIb structure can influence the platelet's functional responses and subsequently the thrombotic risk[14].

Two polymorphisms of the glycoprotein Iba gene that affect the structure have been described and a third one that may lead to altered gene expression of this subunit. In the first polymorphism, a cytosine (C) to thymidine (T) substitution, results in the amino acid methionine[15] in the place of threonine at position 145 and is responsible for the HPA-2 platelet antigen system[16]. This dimorphism is in linkage disequilibrium with a variable number of tandem repeat (VNTR) polymorphism within the macroglycopeptide region of GPIa resulting in the duplication of a 13 amino acid sequence[17]. This last fragment can be present as a single copy or repeated up to four times.

In the third polymorphism of GPIba, a T to C single nucleotide substitution at position 5 from the initiator methionine codon is termed Kozak polymorphism and is thought to alter the translational efficiency of glycoprotein Iba[18]. Moreover, an association between the C-5 allele and increased GPIb / IX/V receptor density has been documented[19-21].

GPIb/IX/V polymorphism and cardiovascular disease

GPIb/IX/V receptor is responsible for the initial platelet adhesion to the subendothelium under high shear stress conditions and several studies have assessed its polymorphisms as potential independent risk factors for myocardial infarction. In a Japanese study of 91 patients with non-fatal myocardial infarction or angina and 105 healthy controls, the Met 145 allele was associated with increased risk of coronary heart disease among a subgroup of patients under the age of 60[22]. At the same time other studies[23,24] failed to confirm this association even when analysis was limited in younger female patients with myocardial infarction[25].

Table 1. The GPIba Met 145/VNTR A or B polymorphism and thrombotic risk.

Positive correlation	Negative correlation
Coronary artery disease	
Murata et al[22]	Ito T et al[24]
Gonzalez-Conejero et al[27]	
Myocardial infarction	
Mikkelson J et al[31]	Hato T et al[23]
	Ardissimo D et al[25]
	Mercier B et al[32]
Cardiovascular disease/stroke	
	Carlsson LE et al[29]
	Carter AM et al[30]

The preliminary data, regarding the role of the VNTR polymorphisms in acute ischaemic events, are also conflicting[26] (Table 1). A limited number of studies have demonstrated an association between Met 145 (VNTR A or B) and risk of cardiovascular disease[15,22,27,28] while others have not found this association[29,30]. In a recent study the HPA-2 Met/VNTR B allele was associated with increased occurrence of myocardial infarction and sudden death in middle age patients[31] (Table 1).

Studies have discrepancies in assessing the risk of GPIb/IX/V polymorphism in different ethnic groups. Among European populations the VNTR B/C genotype was associated with a 2-3 fold increase in risk of coronary artery disease in a Spanish population[27] but no association was detected in a French population[32]. Furthermore, in a prospective study of middle-aged Americans the VNTR C/C genotype was associated with a decreased risk of coronary events[28,33] (Table 1).

The data regarding the role of the Kozak polymorphism of the GPIba variant are also inconclusive. The study by Meisel et al[34] is the first that associated the 5C allele of this polymorphism with an increased risk of unstable angina or ischaemic complication following percutaneous coronary intervention. The same finding was confirmed recently by Kenny et al[35]. In this last study the T-5C polymorphism in GPIb alpha was associated with the risk of MI in a population with unstable angina[35] (Table 2).

Several studies[21,28,36] failed to confirm an association between this dimorphism and clinical risk for arterial thrombosis while others reported a trend

Table 2. Kozak polymorphism and thrombotic risk.

Positive correlation with myocardial infarction	Negative correlation with myocardial infarction
Douglas H et al[37]	Croft S et al[21]
Meisel C et al[34]	Corral J et al[36]
Kenny J et al[35]	Frank MB και συν[38]
	Sperr WR et al[57]

towards protection against myocardial infarction by the 5C allele[37,38]. This discrepancy in the results can be explained partly by differences in the selection of the study population and the choice of the control group (Table 2).

A careful review of the published studies, does not allow us to reach a conclusion regarding the role of GPIb/IX/V polymorphisms in coronary artery disease. Further studies are needed to clarify the role of the polymorphisms of this receptor in coronary artery disease.

Glycoprotein GPIIb/IIIa - Structure and polymorphism

GPIIb and GPIIIa are present in platelet membrane as a heterodimeric complex whose formation requires the presence of divalent cations[39]. Two chains of GPIIb are associated non-covalently with one chain of GPIIIa for the formation of GPIIb/IIIa complex[40,41]. There are approximately 80,000 copies of GPIIb/IIIa per platelet[39] and its major ligands are fibrinogen and vWF either when they are immobilised or in solution after platelet activation.

The genes that encode GPIIb and GPIIIa are both in 17q21 chromosome[42]. A number of point mutations have been described in GPIIb/IIIa's gene and there are data suggesting their interference in the etiology of acute coronary syndromes. Polymorphisms of GPIIb as well as GPIIIa have the ability to produce platelet specific alloantibodies. These antibodies are the main cause of several disorders i.e. post-transfusion purpura and neonatal alloimmune thrombocytopenia[43]. There are at least seven GPIIIa alleles but the most common polymorphism in GPIIIa is described by the human alloantigen system HPA-1 (Pl^A)[39] with frequencies of 97.9% for HPA-1a and 26.5% for HPA-1b in the Caucasian population[44]. Due to the substitution of a cytosine from a thymidine at position 1565 in exon 2 of the GPIIIa gene, the platelet antigen Pl^{A2} variant displays a proline instead of a leucine at position 33[39]. A rare leucine 40/arginine40 polymorphism on platelet glycoprotein IIIa is linked to the human platelet antigen 1b[45]. Four other rare polymorphisms in GPIIIa gene have been described and are related with the alloantigen system HPA-4[46,47], HPA-6[48], HPA-7[49], HPA-8[50].

A thymine (T) to guanine (G) transversion in exon 26 of the glycoprotein IIb gene that encodes an Ile to Ser substitution at amino acid 843 has been reported and is responsible for the expression of the HPA-3 alloantigen system[51].

Glycoprotein IIb Ile 843 Ser and cardiovascular risk

Studies regarding the functional consequences of this polymorphism have yielded conflicting results. Several investigators have observed no effect on *in vitro* platelet aggregation[52] while other reports indicated that platelets with the GPIIb Ser843 allele demonstrate increased *in vitro* platelet aggregation and decreased clot retraction compared to those lacking the allele[53].

Controversial are also the data regarding the role of this polymorphism in coronary artery disease. Reiner et al[54] reported an increased risk of myocardial infarction among women who possessed at least one copy of the GPIIb Ser 843 allele (Table 3). This increased risk was present only in a subgroup of women who had additional cardiovascular risk factors (cigarette smoking, hypercholesterolemia or had a positive family history of early myocardial infarction)[54]. In contrast studies involving male patients from Japan[23] or Central Europe[55-57] failed to detect the same association (Table 3). In addition in a study of 2178 patients with symptomatic coronary disease undergoing coronary stent placement, the Ser 843 allele was not related with the development of coronary stent thrombosis or restenosis[58] (Table 3).

With so many conflicting results from epidemiological studies it is difficult to recognize Iib Ile 843 Ser polymorphism as an important inherited determinant of atherothrombotic risk. The possible

Table 3. GPIIb polymorphism and thrombotic risk.

Positive correlation with myocardial infarction	Negative correlation with thrombosis
Reiner AP et al[54]	Sperr WR et al[57]
	Bottiger C et al[55]
	Hato T et al[23]
	Kroll H et al[56]
	Bottiger C et al[58]

association between the Ser 843 variant and increased risk of arterial thrombotic disease among premenopausal women requires confirmation in larger studies.

Genetics and antiplatelet therapy

Genetic factors are postulated to modulate drug response either in determining efficacy or the risk of side-effects. It has been hypothesised that the clinical efficacy of antiplatelet drugs (i.e aspirin) might be related to PI^A polymorphism. Aspirin inhibition of platelets varies by PI^A genotype[59,60]. In addition, a more specific antiplatelet therapy, GPIIb/IIIa antagonists, have been suggested to have different responses according to PI^A genotype[61-63]. GPIIb/IIIa antagonists bind to the receptor and prevent platelet aggregation to all known agonists but oral GPIIb/IIIa antagonists have been proven to be ineffective and even harmful when administered in patients with acute coronary syndromes[61]. Whether the PI^{A2} variant of the GPIIb/IIIa antagonists is more susceptible to the partial agonist activity induced by smaller ligands such as GPIIb/IIIa antagonists and whether this hypothesis can explain the observed variability in the response to these drugs in humans has yet to be addressed.

Conclusions

Platelet glycoprotein receptors play a primary role in the thrombotic process. They mediate the multiple interactions of platelets with the extracellular matrix and they interfere with coagulation mechanisms. Therefore, platelet glycoprotein polymorphisms may be involved in the process of thrombosis.

The preliminary data regarding the glycoprotein GPIb and GPIIb polymorphisms in ischaemic events as well as in the adverse thrombotic events after coronary interventions are inconclusive and often controversial. The discrepancy in the results of different studies may be explained partly by differences in the study design and the analysis. Many studies have a limited sample size, which is frequently too small to confirm or rule out the presence of a relevant epidemiological association between specific polymorphisms and cardiovascular disease. Moreover, studies differ in ethnicity, bias in selection of patients and controls, plurality in clinical endpoints and variation of environmental factors. Furthermore, as far as the correlations between genes and myocardial infarction are concerned, the true effect of genotype can be masked if mortality rates are the endpoint. Correlations between platelet polymorphisms and environmental risk factors reinforce this. Moreover, several genes are in linkage disequilibrium with other genes and simultaneous studies of several genes may reveal associations that at present seem to be weak. Regarding the correlations between genes and myocardial infarction, the true effect of genotype can be masked by whether patients died of acute coronary syndromes or when only survivors are included in the studies.

Considering that atherosclerosis is a multifactorial disease it is extremely difficult to conclude that genetic inheritance will be enough to explain the interindividual variations by itself. Correlations already noticed between platelet polymorphisms and environmental risk factors reinforce this assumption. It is difficult to arrive at a definite answer for the role of platelet polymorphisms and especially for GPIb and GPIIb polymorphism, as present reports are inconsistent. Understanding the interaction of platelet glycoprotein polymorphisms with cardiovascular risk factors and endovascular procedures may also influence treatment strategies targeting a specific susceptibility gene implicated in coronary thrombosis. Further studies are needed to clarify the potential association between platelet polymorphisms, coronary artery disease and myocardial infarction.

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[General]

The Merck Manual of Diagnosis and Therapy
Section 11. Hematology And Oncology
Chapter 132. Thrombotic Disorders

Topics

[General]

navigation help

Thrombotic disorders: Diseases characterized by formation of a thrombus that obstructs vascular blood flow locally or detaches and embolizes to occlude blood flow downstream (thromboembolism).

Thrombi are mechanical masses that form within the cardiovascular system on denuded endovascular or prosthetic flow surfaces. They are composed of insoluble fibrin, deposited platelets, accumulating WBCs, and entrapped RBCs in variable flow-dependent patterns.

Thrombus formation is a multifactorial process involving many mutually interactive genetic and environmental factors. Thrombotic predisposition is usually identified clinically. The most important features are family history, recurrence, young age, severity of provocation, and unusual sites of thrombosis.

Suspected arterial or venous thrombosis or thromboembolism requires objective confirmation. Angiography is the diagnostic reference standard. However, ultrasonography performed by skilled personnel is suitable for superficial vessels and for cardiac assessment.

Of patients with venographically proven spontaneous deep vein thrombosis, 25 to 50% have a genetic predisposing factor. A genetically impaired anticoagulant mechanism (eg, factor V resistance to activated protein C, hyperhomocysteinemia, protein C deficiency, protein S deficiency, antithrombin deficiency, defective fibrinolysis), when combined with a thrombotic stimulus (eg, surgery, pregnancy, oral contraceptive use, antiphospholipid antibodies), is sufficient to develop a venous thromboembolism. Persons with more than one abnormality experience thrombosis earlier, more frequently, and more severely than those with single defects.

Antithrombotic therapy involves the use of thrombolytic drugs, antiplatelet drugs, and anticoagulants. Thrombolytic drug therapy is the first consideration when formulating an antithrombotic strategy because thrombolytic drugs can remove an established thrombus. Subsequent antithrombotic therapy varies depending on whether the venous or arterial circulatory system is involved; the size and location of the involved vessels; the risks of extension, embolization, or recurrence; and the relative antithrombotic benefits and hemorrhagic risks.

Mechanical measures for restoring vascular patency include balloon catheter and surgical

embolectomy. Indications and complications related to antithrombotic regimens for specific disorders (eg, MI, venous thrombosis, pulmonary embolism, cerebrovascular accident, prosthetic heart valves, arterial embolism) are summarized elsewhere in *The Manual.*

Factor V Resistance to Activated Protein C

Resistance to activated protein C (APC) results from the genetic point mutation factor V Leiden. This defect is the most common genetic risk factor associated with familial predisposition to venous thrombosis. Its prevalence in European populations is 5%, but it rarely occurs in native Asian or African populations. APC resistance phenotype is found in 20 to 60% of patients with spontaneous venous thrombosis. Mutated factor V is activated by thrombin or factor Xa in the usual way, but its inactivation by APC is impaired.

Hyperhomocysteinemia

Plasma homocysteine levels are elevated tenfold or greater in homozygous cystathionine β-synthase deficiency; these patients are at great risk of arterial and venous thromboembolism. Hyperhomocysteinemia is also strongly correlated with atherosclerotic thrombosis (including coronary artery disease--see Ch. 201). Mild cases occur in heterozygous cystathionine β-synthase deficiency and in other abnormalities of folate metabolism, including methyltetrahydrofolate dehydrogenase deficiency. Homocysteine levels may be normalized by dietary supplementation with folate and, if needed, pyridoxine, but this has not been shown to reduce the risk of thrombosis.

Protein C Deficiency

Heterozygous deficiency of plasma protein C is transmitted in an autosomal dominant fashion, with a prevalence of 0.2 to 0.5%; about 75% of persons with this defect will experience a venous thromboembolism (50% by age 50 yr). Homozygous or doubly heterozygous deficiency presents in the newborn as purpura fulminans or disseminated intravascular coagulation (DIC) and is fatal without replacement therapy and anticoagulation. Acquired decreases are observed in patients with liver disease, severe infection, or DIC; during cancer chemotherapy (including L-asparaginase); after surgery; and with warfarin therapy. Laboratory screening involves use of functional assays. In patients with symptomatic thrombosis, it is important to initiate antithrombotic therapy with full heparin anticoagulation before beginning warfarin because of the danger of skin necrosis. Warfarin occasionally causes thrombotic skin infarction by lowering protein C levels before decreasing the majority of vitamin K-dependent clotting factors.

Protein S Deficiency

Heterozygous deficiency of plasma protein S is similar to protein C deficiency in genetic transmission, prevalence, incidence, and laboratory screening. Acquired deficiencies are observed during pregnancy, severe infection, DIC, HIV, oral contraceptive use, and warfarin therapy and after L-asparaginase administration. The treatment precautions are the same as those for protein C deficiency.

Antithrombin Deficiency

Heterozygous deficiency of plasma antithrombin is inherited in an autosomal dominant fashion with a prevalence of about 0.2 to 0.4%; about half of these persons experience venous thrombotic episodes. Acquired deficiencies in antithrombin levels are observed in patients with acute thrombosis, DIC, liver disease, or nephrotic syndrome and during heparin therapy, estrogen therapy (including contraceptive use), or L-asparaginase therapy. Laboratory screening should involve use of an antithrombin-heparin cofactor assay because it detects all different subtypes. Oral anticoagulation is highly effective prophylaxis for patients who have experienced or are at risk of thrombosis.

Defective Fibrinolysis

Inherited disorders of plasminogen--decreased tissue plasminogen activator levels or increased plasminogen activator inhibitor levels--are rare. They have been associated with unexplained venous thromboembolism in younger patients. Screening produces many false-positive and false-negative results. Possible hereditary fibrinolytic abnormalities should be investigated in a research setting.

Phospholipid Syndrome

(Antiphospholipid Syndrome; Antiphospholipid Antibodies; Anticardiolipin Antibodies; Lupus Anticoagulant)

This syndrome includes thromboembolism (particularly involving CNS vasculature), thrombocytopenia, and fetal loss in association with autoimmune antibodies directed against phospholipid membrane constituents. In vitro clotting tests are prolonged. The mechanism of action may involve antibody-induced platelet activation, yielding phosphatidylserine-rich procoagulant surfaces and thrombocytopenia.

Atherosclerosis

(See also Ch. 201.)

Patients with symptomatic atherosclerosis are at significant risk of stroke, MI, and peripheral artery occlusion, which primarily develop at sites of preexisting stenosis. Atherosclerotic plaques rupture and expose tissue factor-rich plaque contents to blood. This initiates thrombin-mediated, platelet-rich thrombus formation. Increases in fibrinogen levels correlate with thrombotic events. High levels may be an independent risk factor for arterial thromboembolism or a nonspecific inflammatory marker of ruptured plaques.

Thrombocytosis

In patients undergoing invasive vascular procedures (eg, saphenous vein bypass grafts, small-caliber vascular grafts), the frequency of thrombotic complications correlates with the peripheral platelet concentration. However, in the absence of vascular disruption, there is little relationship between arterial thrombosis and thrombocytosis, even if peripheral platelet counts are very high, especially in young asymptomatic persons.

Other Predisposing Factors

Stasis is associated with increased venous thromboembolism in surgery, orthopedic or paralytic immobilization, congestive heart failure, pregnancy, varicosities, and obesity.

Tissue injury from trauma and surgery increases the frequency of venous thromboembolism. Coagulation serine proteases are activated, and platelets are triggered by the exposure of tissue factor to flowing blood.

Neoplastic cells may activate platelets, coagulation proteases, or both by secreting adenosine diphosphate-like activating substances and expressing tissue factor on exposed membrane surfaces. The resultant circulating activated species trigger thrombus formation at vulnerable sites of vascular stasis or injury. Malignancies associated with increased thrombotic predisposition include promyelocytic leukemia and tumors involving lung, breast, prostate, GI tract, and other sites. Advanced metastatic disease may induce DIC. An extensive search to identify the underlying disease is not indicated because the malignancy is usually evident.

When exposed to flowing blood, **chronic inflammatory processes** that are associated with tissue factor expression by monocytes or macrophages may initiate thrombosis.

Cardiovascular prosthetic devices may induce chronic monocyte or macrophage accumulation in relation to their flow surfaces, enhancing the risk of thrombotic device failure.

Oral contraceptive drugs that contain estrogen are associated with venous thromboembolism. These patients often have a coexisting genetic factor predisposing them to venous thrombosis, particularly factor V resistance to APC or deficiency of protein C or S.

HEMOSENSE

Thrombotic Disorders

The human body has a complex mechanism that causes blood to clot if a wound occurs. Under normal circumstances this is a desirable response that enables the body to heal itself, but under certain clinical conditions, called "thrombotic disorders", this same mechanism can cause an unwanted clot or "thrombus" that can be life threatening. These conditions are:

Atrial Fibrillation: An abnormality in the heart rhythm, (i.e. irregular heartbeat) which can cause clots to form in the walls of the atria (heart).

Mechanical Heart Valves: Surgical replacement of faulty heart valves with mechanical valves. The body can react to the "foreign" mechanical valve and start the clotting process.

Heart Attack (Myocardial Infarction): Heart muscle tissue damaged from a heart attack can cause clots to form within the heart.

Unstable Angina: Chest discomfort and spasms due to inadequate supplies of oxygen to the heart caused by a narrowing of the coronary arteries. These spasms help form unwanted blood clots.

Deep Vein Thrombosis (DVT): A deep vein within the muscle of the thigh, leg or pelvis that has formed a clot.

Pulmonary Embolism: A clot that breaks off from the deep veins of the muscle and travels to the arteries of the lungs. This is referred to as an embolus.

Acute Ischemic Stroke: A clot originating in another part of the body travels to the brain, causing a sudden loss of blood supply.

These conditions must be managed through the use of an oral anticoagulant, a drug which decreases the clotting ability of the blood so the unwanted clots are prevented.

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Acute Coronary Syndromes: New Developments in Pharmacological Treatment Strategies

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Acute coronary syndromes—unstable angina, non–Q-wave myocardial infarction (MI), and Q-wave MI—share the pathophysiological feature of thrombosis due to disruption of atherosclerotic plaque. Advances in understanding of the pathophysiology of acute coronary syndromes, in particular the role of platelets in thrombus formation, have led to the development of novel therapeutic agents and treatment strategies that hold promise for better clinical outcomes.

For advanced practice nurses, this rapidly evolving field presents an informational challenge. Optimal patient care requires fluency with treatment strategies, but it is difficult to monitor the numerous studies that are ongoing at any given time and even more challenging to evaluate their significance to daily clinical practice and specific patient populations. This article reviews the pharmacology of new treatment agents and some of the evolving treatment paradigms for unstable angina and acute myocardial infarction, focusing on appropriate use of medications for acute coronary syndromes and practical implications for the nurse clinician.

Physiology and Pharmacology

Coagulation Cascade

Activation of platelets can occur when a blood vessel is damaged. Platelets aggregate causing an occluding clot that can halt the flow of blood.[1] A blood clot or thrombus is a complex structure of these aggregated platelets bound together with fibrin strands that are produced by the action of thrombin on fibrinogen.[2,3] To open an occluded vessel, pharmacological therapy may be used, with or without mechanical intervention, to inhibit blood coagulation (anticoagulants), stimulate lysis of a dangerous thrombus (fibrinolytics), or inhibit platelet function (platelet aggregation inhibitors)[4] (Figure 1).

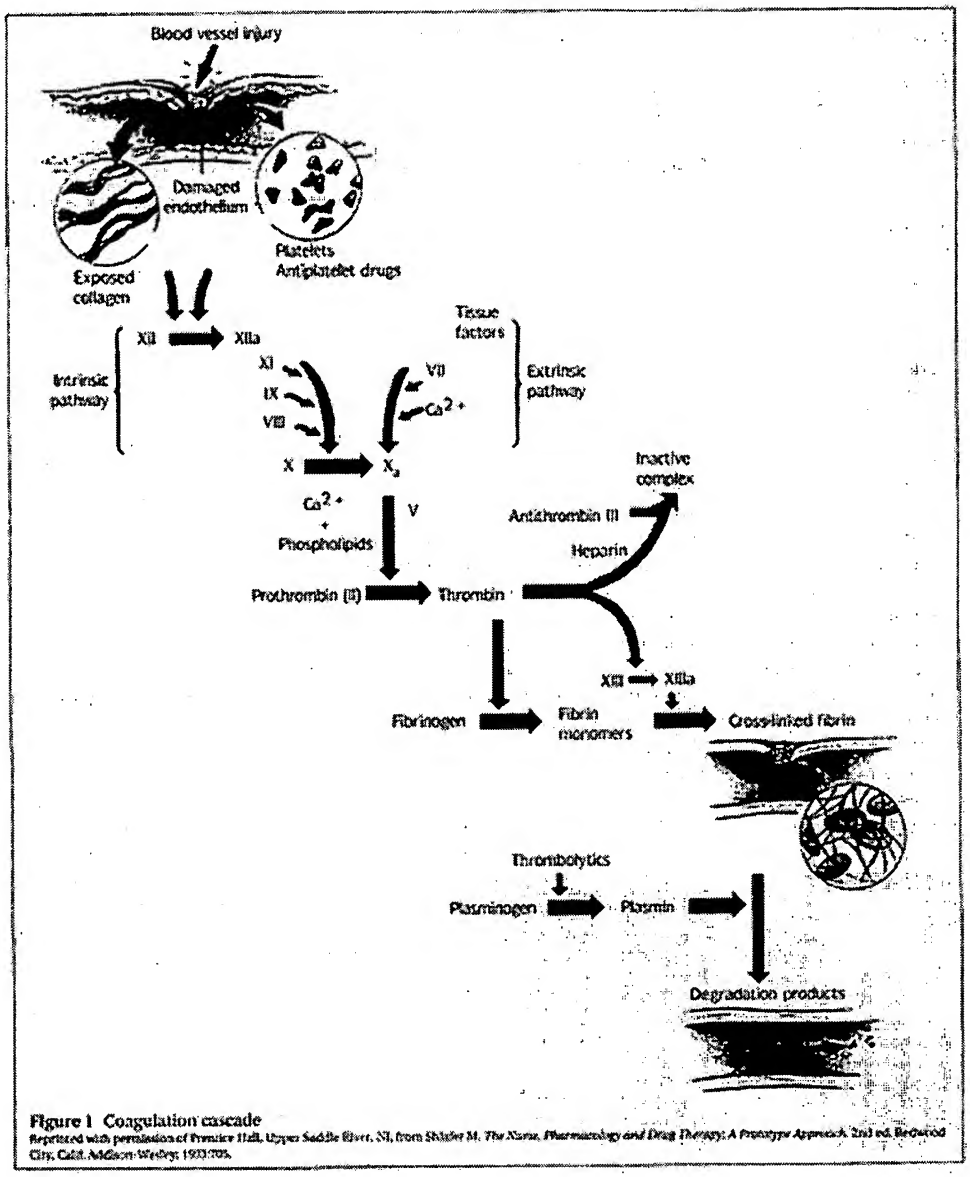

Figure 1 Coagulation cascade

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For example, the anticoagulant heparin binds to the plasma glycoprotein, antithrombin III (ATIII), and inhibits thrombin; fibrinolytic agents lyse clots through the activation of plasminogen; and platelet glycoprotein IIb/IIIa (GpIIb-IIIa) inhibitors inhibit platelet function by blocking the GpIIb-IIIa receptor, the final common pathway of platelet aggregation.[5] Recent developments in the use of these therapies have resulted in improved outcomes for patients with unstable angina and acute MI.

Pharmacology of Antiplatelet, Thrombolytic and Anticoagulation Agents

Antiplatelet Agents

The following antiplatelet agents—GpIIb-IIIa antagonists—are currently available: abciximab, eptifibatide, and tirofiban. Glycoprotein IIb/IIIa antagonists inhibit platelet aggregation by blocking the GpIIb-IIIa receptor, a surface receptor involved in the final common pathway of platelet aggregation.[5] Platelet adherence to the endothelial cells lining blood vessels is the initial response of hemostasis that begins after platelet stimulation of plasma coagulation factors. Interruption of platelet adherence by GpIIb-IIIa antagonists decreases thrombi development and prevents arterial vessel occlusion.

Abciximab is a monoclonal antibody antigen-binding fragment (Fab) and was the first of these antiplatelet agents to be developed; eptifibatide and tirofiban are small molecules that were subsequently developed to interact with the same GpIIb-IIIa receptor but have a longer duration of action than abciximab.[6]

Fibrinolytic Agents

The following fibrinolytic agents are currently available: streptokinase, urokinase, alteplase, anistreplase, and reteplase. Fibrinolytic drugs are used to restore artery patency after infarct in patients diagnosed with acute MI. These drugs all work by converting inactive plasminogen to active plasmin, an enzyme responsible for degradation of fibrin clots. Streptokinase and urokinase are the first generation of fibrinolytic agents currently in use. Streptokinase is a protein produced by beta-hemolytic streptococci; urokinase is isolated from human renal cells and is the drug of choice for treatment of pulmonary emboli.

However, urokinase is not currently readily available because of manufacturing problems. Newer fibrinolytic agents, (alteplase, anistreplase, and reteplase), are more clot selective than streptokinase and urokinase, ie, they have more activity on clot plasminogen than circulating plasminogen. Anistreplase is a streptokinase derivative formulated to be more effective than streptokinase because of greater fibrin specificity. Alteplase is also fibrin specific, with less effect on circulating plasmin resulting in fewer systemic effects.

An accelerated dosing regimen recommended for alteplase (accelerated tissue plasminogen activator [tPA]) improves fibrinolysis but is associated with higher morbidity, ie, hemorrhagic stroke. Reteplase is the newest fibrinolytic drug. It is an alteplase derivative with distinct advantages: a longer half-life allowing administration as a double bolus, and ease of administration resulting in a decreased door-to-drug time. Reteplase does have a lower affinity for fibrin, which may lead to an increased incidence of reocclusion. Consequently, this agent has been combined with GpIIb-IIIa inhibitors in recent clinical trials.[7]

Anticoagulants: Low Molecular Weight Heparins

Enoxaparin and dalteparin, low molecular weight heparins, are isolated from standard heparin preparations and have a similar mechanism of action to heparin, with some distinct advantages. Anticoagu-lant activity follows binding of the low molecular weight heparins to antithrombin III, which results in further binding and inactivation of coagulation factors IIa (thrombin) and Xa. Enoxaparin has identical inhibitory activity for factors IIa and Xa; dalteparin is effective in inhibiting factor Xa only. Advantages of these agents include improved bioavailability of the drugs, a longer plasma half-life, and less bleeding compared with heparin. Onset of action of these agents is 3 to 5 hours and may continue for 24 hours after subcutaneous injection.

Unstable Angina

Standard Treatment

The 2 primary approaches in the treatment of unstable angina consist of conservative management or more aggressive early intervention with percutaneous or surgical revascularization. Until recently, medical therapy primarily has consisted of use of aspirin and/or standard unfractionated heparin. There has been considerable debate about whether early intervention in unstable angina patients improves clinical outcome. Initial studies of conservative management versus early intervention provided conflicting results.

In the Thrombolysis in Myocardial Infarction IIIb (TIMI IIIb) study,[8,9] the rate of death or nonfatal MI was slightly lower in patients with early intervention. The rate of death or nonfatal MI was similar in the 2 groups in the Organization to Assess Strategies for Ischemic Syndromes (OASIS) registry,[9,10] and lower among conservatively treated patients in the Veterans Affairs Non–Q-Wave Infarction Strategies in Hospital

(VANQWISH) trial.[9,12] Each of these trials demonstrated improvements in nonfatal events with early intervention: TIMI IIIb and VANQWISH show lower early rates of MI among patients receiving early intervention and the OASIS registry indicates improved outcome among such patients with regard to incidence of refractory angina and hospital admission for unstable angina at both early and 6-month follow-up.

It is important to note, however, that these studies were performed prior to improvements in percutaneous procedures in standard use, prior to the advent of widespread use of coronary stenting, and prior to the development and use of GpIIb-IIIa inhibitors as adjunctive treatment to intervention.

New Approaches

Glycoprotein IIb/IIIa Blockade

Recognition of the critical role of platelets in thrombus formation and the role of the platelet GpIIb-IIIa receptor as the final common pathway for platelet aggregation led to the development of the GpIIb-IIIa inhibitors abciximab, tirofiban, and eptifibatide.[12] These potent agents represent a major advance in both surgical intervention and medical treatment of unstable angina and non–Q-wave MI. The first major trials to show the benefit of GpIIb-IIIa inhibitor treatment for acute coronary syndrome patients were those of the agent abciximab in patients undergoing percutaneous coronary intervention.

In the Evaluation of 7E3 (abciximab) in Preventing Ischemic Complications (EPIC) trial,[13,14,15] in high-risk patients (severe unstable angina, evolving MI, or high-risk coronary morphological characteristics), abciximab treatment was associated with a 35% reduction in the 30-day rate of the composite end point of death, nonfatal MI, or urgent revascularization compared with aspirin and heparin alone. Significant benefit persisted at 6 months and 3 years after treatment; at 3 years, abciximab treatment was associated with a 60% reduction in mortality among patients with unstable angina or evolving MI.

In the subsequent Evaluation in PTCA (percutaneous transluminal coronary angioplasty) to Improve Long-Term Outcome with abciximab GpIIb-IIIa blockade (EPILOG) trial16 in patients across all risk strata, abciximab treatment produced a 56% reduction in the composite end point at 30 days. One component of the ongoing Global Use of Strategies To Open Occluded Coronary Arteries IV Acute Coronary Syndrome (GUSTO IV ACS) trial is assessing the effects of 2 different abciximab infusion lengths (24 and 48 hours) as medical management in patients with unstable angina and non–Q-wave MI, with a primary outcome measure of death and MI at 48 hours.

Most recently, the EPISTENT (Evaluation of Platelet IIb/IIIa Inhibitor for STENTing) trial has shown that adjunctive use of abciximab with coronary stenting produced a 52% reduction in the 30-day composite end point rate compared with stenting alone.[17] This study is of particular importance given the fact that the majority of coronary interventions now involve stent placement. Abciximab was associated with a 58% reduction in mortality at 1 year compared with stenting (1.0% for patients treated with abciximab vs 2.4% for patients receiving placebo, P=.03).[12] A meta-analysis of the effects of abciximab and other GpIIb-IIIa inhibitors in percutaneous coronary intervention trials has demonstrated overall significant benefits in reduction of rates of mortality, MI, or urgent revascularization at 30 days (Figure 2).[12]

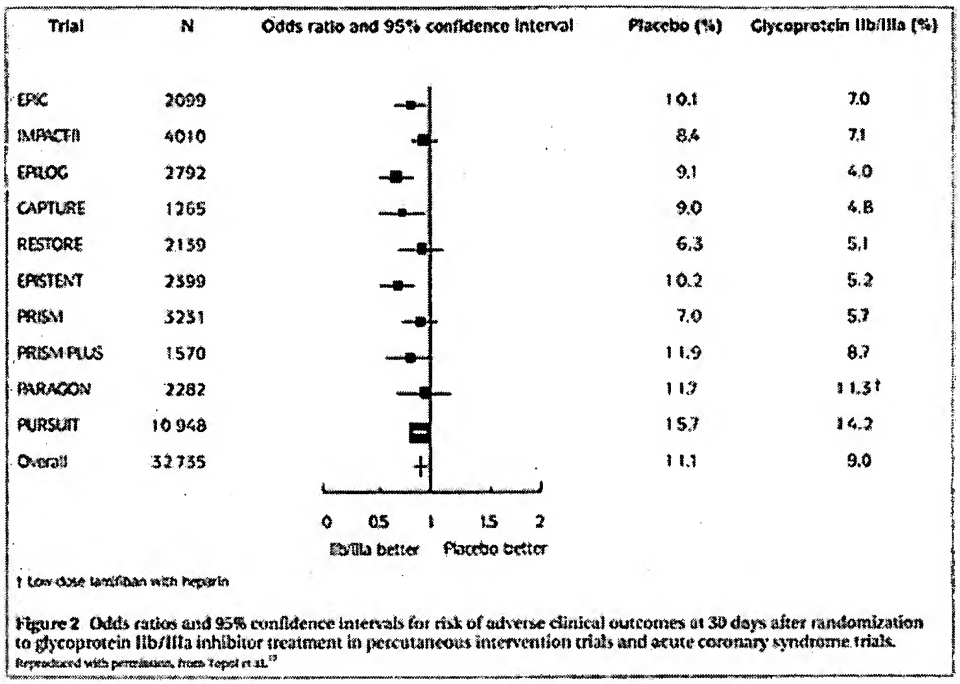

Figure 2 Odds ratios and 95% confidence intervals for risk of adverse clinical outcomes at 30 days after randomization to glycoprotein IIb/IIIa inhibitor treatment in percutaneous intervention trials and acute coronary syndrome trials.
Reproduced with permission, from Topol et al.[12]

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Benefits of GpIIb-IIIa inhibitor treatment as medical management have also been demonstrated in major trials. In the Platelet Receptor Inhibition in Ischemic Syndrome Management (PRISM) trial of tirofiban in patients with unstable angina, tirofiban treatment was associated with a 32% reduction in the composite end point of death, MI, or refractory ischemia at 48 hours.[18] In the Platelet Receptor Inhibition in Ischemic Syndrome Management—Patients Limited by Unstable Signs and Symptoms (PRISM-PLUS) trial in higher-risk patients, tirofiban treatment was associated with a 28% reduction in the 7-day composite end point rate, with a significant preventive benefit also observed at 30 days.[19]

In the Platelet IIb/IIIa Underpinning the Receptor for Suppression of Unstable Ischemia Trial (PURSUIT), eptifibatide treatment was associated with a significant reduction in the 30-day composite end point of death or MI.[20] As with use of GpIIb-IIIa inhibitors in the setting of coronary intervention, meta-analysis of the effects of these agents in medical management of acute coronary syndromes indicates significant reductions in incidence of death, MI, or urgent revascularization at 30 days (Figure 2).[12]

The C7E3 Fab AntiPlatelet Therapy in Unstable Refractory Angina (CAPTURE) trial of abciximab in patients with refractory unstable angina assessed the effects of GpIIb-IIIa inhibitor treatment beginning 18 to 24 hours prior to percutaneous angioplasty and continuing for 1 hour after the procedure.[21] Although CAPTURE is an intervention trial, it differs from other intervention trials in that GpIIb-IIIa inhibitor treatment was initiated well before intervention; because many of the patients in the medical management trials subsequently underwent coronary interventions after initiating GpIIb-IIIa treatment, CAPTURE is also similar to these latter trials in some respects.

In CAPTURE, abciximab was associated with a 71% reduction in MI in the pre-angioplasty period and a 53% reduction during and for 24 hours after angioplasty; it was also associated with a 29% reduction in the primary outcome measure of death, MI, or urgent revascularization at 30 days.

A substudy of the CAPTURE trial has provided important insights regarding patient

selection for GpIIb-IIIa blockade.[22] In patients with unstable angina, elevated serum levels of troponin I and troponin T are recognized as important markers of necrotic injury caused by thrombus particles moving downstream from coronary artery plaques.[23,24,25] Among those CAPTURE patients with troponin T levels less than 0.1 ng/mL, the composite rate of death and nonfatal MI was 23.9%, compared with 7.5% in patients without elevated troponin T ($P<.001$). In addition, the use of abciximab among patients with elevated troponin T improved outcomes significantly, indicating that GpIIb-IIIa antagonists may resolve these distal thrombi and reduce risk of further events. The CAPTURE investigators concluded that serum troponin T levels provide an important means of identifying patients with refractory unstable angina who can benefit from GpIIb-IIIa inhibition.[22]

Low Molecular Weight Heparin

Other recent advances in treatment of unstable angina and non–Q-wave MI include demonstration of the superiority of low molecular weight heparin to standard unfractionated heparin. Several low molecular weight heparins, which are administered subcutaneously and are, therefore, considerably easier to use than standard heparin, are currently available for use in the United States.

Comparison of the low molecular weight enoxaparin and unfractionated heparin in the Efficacy and Safety of Subcutaneous Enoxaparin in Unstable Angina and Non–Q-Wave Myocardial Infarction (ESSENCE) trial showed that the former reduced the risk of the composite end point of death, MI, or recurrent angina by 15% at 30 days.[26] In the Fragmin During Instability in Coronary Artery (FRISC) trial, dalteparin treatment resulted in a 63% decrease in rate of the primary outcome measure of death or MI at 6 days compared with placebo.[27]

The subsequent Fragmin During Instability in Coronary Artery II (FRISC II) trial assessed 3-month treatment with dalteparin versus placebo. Dalteparin was associated with a 47% reduction in death or MI at 30 days, with a significant benefit persisting at 60 days. However, the difference was no longer significant at 3 months. [28]

Several other promising studies are examining the role of low molecular weight heparins as adjunctive therapy to GpIIb-IIIa inhibitors. Early results from the National Investigators Collaborating on Enoxaparin (NICE) 4 study showed that enoxaparin combined with abciximab was associated with a low bleeding risk when used in conjunction with percutanous intervention.[29] The incidence of thrombocytopenia was also reduced when enoxaparin was used rather than standard unfractionated heparin.[30] A substudy of the ongoing GUSTO IV ACS trial will further assess the combination of abciximab with the low molecular weight heparin, dalteparin.

Acute Myocardial Infarction

Standard Treatment

Fibrinolytic Therapy

The 2 main approaches to treatment of acute MI are fibrinolytic therapy and percutaneous coronary intervention. Fibrinolytic therapy with agents such as alteplase (tPA) and reteplase (recombinant tissue plasminogen activator [rPA]) remains a mainstay of treatment for MI. Early trials demonstrated that treatment with fibrinolytic agents could play a significant role in reducing mortality. A 1994 meta-analysis of outcomes in controlled fibrinolytic trials involving more than 58 000 patients showed that fibrinolytic therapy was associated with a highly significant 18% reduction in 35-day mortality, with benefit observed regardless of such variables as age, sex, blood pressure, heart rate, or history of MI or diabetes.[31]

Fibrinolytic therapy was associated with high rates of mortality during days 0 to 1,

particularly in patients presenting at more than 12 hours after symptom onset, with this early risk being outweighed by the much greater subsequent benefit. Treatment was associated with highly significant mortality reductions of approximately 30 per 1000 in patients presenting within 6 hours of onset, and 20 per 1000 in those presenting within 7 to 12 hours, with a statistically uncertain reduction of 10 per 1000 observed in those presenting within 13 to 18 hours.

The Global Utilization of Streptokinase and Tissue Plasminogen Activator (tPA) for Occluded Coronary Arteries (GUSTO I) study established the superiority of accelerated tPA over prior standard fibrinolytic therapy with streptokinase.[32] In this trial, accelerated tPA plus intravenous (IV) heparin resulted in a highly significant 14% relative reduction in 30-day mortality compared with streptokinase plus either subcutaneous or IV heparin, and reduced mortality compared with combined tPA and streptokinase. Accelerated tPA, however, was associated with a higher incidence of hemorrhagic stroke (0.72% for patients treated with accelerated tPA vs 0.49% for patients treated with streptokinase and subcutaneous heparin and 0.54% for patients treated with streptokinase and IV heparin, P=.03).

An angiographic substudy of the GUSTO trial was performed to determine whether the speed and degree of reestablishment of coronary patency determine clinical outcome.[33] It was found that accelerated tPA treatment was associated with a higher rate of patency at 90 minutes (81%) compared to the other treatments (54% for streptokinase plus subcutaneous heparin, 60% for streptokinase plus IV heparin, and 73% for the combination of tPA and streptokinase). Flow was normal in the artery at 90 minutes in 54% of the tPA recipients compared with less than 40% of patients in the other groups.

By 180 minutes, patency rates were similar in all 4 groups. Left ventricular function was found to be associated with patency rate at 90 minutes, with function being superior in those patients receiving tPA and in those receiving any treatment who had normal flow at 90 minutes. Mortality was lowest (4.4%) in those with normal flow at 90 minutes and greatest (8.9%) in those with no flow at 90 minutes.

These findings indicate that more rapid and complete return of flow in the coronary artery results in improved ventricular function and reduced mortality, and provide an explanation for the superiority of accelerated tPA treatment in the GUSTO I trial. This study was used to establish the working model for an important standard of reperfusion therapy. Often referred to as the open artery hypothesis, this theory holds that clinical outcomes in the post-MI period can be greatly improved by early and significant restoration of coronary blood flow.

Percutaneous Coronary Intervention

Percutaneous coronary intervention has also emerged as an effective method of treating acute MI, with evidence suggesting that it is associated with improved outcomes compared with fibrinolytic therapy. A recent quantitative review evaluated results of trials comparing primary coronary angioplasty with thrombolytic therapy with streptokinase, standard-dose tPA infusion, or accelerated tPA infusion.[34] The authors found that angioplasty was associated with a 34% reduction in 30-day mortality and a significant reduction in the rate of death and nonfatal MI (7.2% vs 11.9%).

The data also indicate that percutaneous intervention is associated with a reduction in the significant risk of stroke that has been observed with available thrombolytic regimens. In this review of comparative trials, angioplasty was associated with significant reductions in total strokes (0.7% vs 2.0%) and hemorrhagic strokes (0.1% vs 1.1%).

Treatment delays have proved to be a confounding factor in implementing percutaneous intervention in acute MI, as these procedures are most effective when performed immediately on presentation. A recent analysis of more than 27 000 MI patients

undergoing primary angioplasty within 24 hours of symptom onset showed that delayed treatment (door-to-balloon time of less than 2 hours) was associated with a highly significant 40% to 60% increase in mortality.[35] This report further underscores the need for continuing patient education efforts to encourage MI suspects to seek medical attention when symptoms initially occur. Additional emphasis has recently been placed on the importance of development of treatment protocols for rapid and appropriate management of these patients.

New Approaches to Treating MI

Fibrinolytic Therapy

Reteplase. Fibrinolysis continues to be a mainstay of acute MI treatment because of its universal applicability and availability. Potential advances include the availability of rPA, the first new fibrinolytic agent to be introduced within the past 10 years. This agent represents a modification of the tPA molecular structure (absence of the kringle-1, finger, and growth factor domains of tPA) resulting in a smaller molecular weight and different in vivo and in vitro characteristics. Unlike alteplase, reteplase does not require weight-based dosing adjustment and is suitable for double-bolus dosing.

This characteristic is important because evidence increasingly suggests that medication errors occur more frequently with agents requiring weight-adjusted infusion, and that such errors predict poor clinical outcomes in patients with acute MI. In the GUSTO I study, for example, protocol deviations were associated with increased risk of mortality at both 24 hours and 30 days after fibrinolysis.[36] Another study found that tPA treatment was associated with significantly more intravenous catheter complications than was rPA treatment.[37]

Patients in the rPA group also received treatment 17 minutes faster than those treated with tPA, with door-to-drug time averaging 33 minutes for rPA versus 51 minutes for tPA. Further evidence from the Intravenous nPA for Treatment of Infarcting Myocardium Early (InTime) II study shows that 30-day mortality and incidence of intracranial hemorrhage rates may be increased in patients receiving tPA compared with those receiving deletion mutant of tissue plasminogen activator (nPA).[38] Compared with the experimental bolus agent, lanoteplase (nPA), which was administered correctly 94% of the time, tPA was given correctly only 80% of the time.

As discussed, speed of reperfusion is an important factor in the management of acute MI, and several trials have evaluated rPA in this context. The Reteplase (rPA) versus Alteplase Patency Investigation During Myocardial Infarction (RAPID) 2 study compared double-bolus rPA with front-loaded, accelerated tPA in 324 patients with MI, with the primary end point of infarct artery patency at 90 minutes.[39] The rPA treatment resulted in greater patency at 90 minutes and at 60 minutes. Patients receiving rPA underwent significantly fewer subsequent acute interventions and had a nonsignificantly reduced 35-day mortality rate, with the 2 groups having a comparable rate of hemorrhagic stroke (1.2% vs 1.9%).

The efficacy of rPA was confirmed in the subsequent Global Use of Strategies to Open Occluded Arteries III (GUSTO III) trial in more than 15 000 patients.40 In this trial, however, no differences between double-bolus rPA and accelerated tPA were observed with regard to 30-day mortality (7.47% vs 7.24%) or incidence of stroke (1.64% vs 1.79%).

Combination Therapy. Recognition of the role of platelets in thrombus formation has led to advances in approaches to lysing clots. The plasminogen activators are capable of lysing the fibrin component of clots but do not affect the platelet core of the thrombus.[41] Recent studies have focused on the potential for combining fibrinolytic drugs with potent GpIIb-IIIa agents that prevent platelet aggregation. Also supporting a combination of

these agents is emerging evidence that myocardial malperfusion occurs frequently, even in patent arteries, as a result of microvascular obstruction due to platelet microemboli or microthrombi.[42]

Lysis of the fibrin component of a clot with a fibrinolytic agent alone may result in disruption of thrombus fragments that may then become lodged in the microcirculation downstream, resulting in reduced coronary blood flow. Therefore, these tiny thrombus particles composed of aggregated platelets can ultimately hamper myocardial perfusion even in arteries with normal flow (described as grade 3 flow in the TIMI study). Fibrinolysis also results in increased levels of free thrombin, which is a potent platelet activator. With microcirculatory spasm resulting from release of platelet products and reduced flow following ischemic insult, activated platelets would be expected to aggregate, resulting in additional obstruction.

Additional strong clinical evidence of the benefit of GpIIb-IIIa inhibitor treatment in patients with acute MI was provided by a study in which 200 patients with acute MI underwent percutaneous coronary angioplasty or stent placement and received abciximab or conventional therapy with heparin.[43] Whereas both groups exhibited comparable peak flow immediately following recanalization, the abciximab group had significantly greater increases in flow at 14 days.

Abciximab-treated patients also had significantly greater increases in wall motion index and a significantly greater reduction in the number of hypokinetic chords, indicating an overall improvement in myocardial function. The 30-day rate of death, MI, or reintervention was reduced by 80% with abciximab treatment. These findings suggest that in addition to helping to achieve coronary artery patency, GpIIb-IIIa inhibitor treatment may prevent microcirculatory microembolization and obstruction, thereby improving myocardial function and salvage.

The rationale for combining fibrinolytic agents with GpIIb-IIIa inhibitor treatment includes the potential for enhanced reperfusion efficacy because both the fibrin and platelet components of the thrombus are targeted. Because lower doses of fibrinolytic agents can be used—thereby reducing hemorrhagic risk—the potential for enhanced safety also exists. Early experience with this combined approach includes the Thrombolysis and Angioplasty in Myocardial Infarction 8 (TAMI 8)44 and Integrilin to Minimize Platelet Aggregation and Coronary Thrombosis—Acute Myocardial Infarction (IMPACT-AMI) studies.

In TAMI 8, 70 patients with acute MI received standard aspirin, heparin, and tPA with or without ascending bolus doses of abciximab at 3, 6, and 15 hours after the start of tPA infusion. Abciximab-treated patients had improved rates of infarct-related coronary patency (92% vs 56%), reduced recurrent ischemia (13% vs 20%), and a reduced incidence of major bleeding (15% vs 5%). In the IMPACT-AMI study,[45] 132 patients received ascending doses of the GpIIb-IIIa inhibitor eptifibatide or placebo, with accelerated tPA, aspirin, and heparin.

An additional 48 patients were randomized to the highest eptifibatide dose from the first phase or placebo, along with tPA. Among those patients receiving the highest eptifibatide dose, normal flow at 90 minutes occurred in 66%, compared with 39% of placebo recipients; the eptifibatide recipients also exhibited a shorter median time to ST-segment recovery (65 vs 116 minutes). The groups had comparable rates for the composite end point of death, reinfarction, stroke, urgent revascularization, new heart failure, or pulmonary edema. Rates of severe bleeding were similar (4% vs 5%). These early studies suggested that both the frequency and speed of reperfusion could be enhanced by combination treatment without increasing hemorrhagic risk.

Both rPA and tPA were evaluated in combination with abciximab in the more recent TIMI

14 trial.[46,47,48] This study compared the use of full-dose rPA or tPA with the use of abciximab and low-dose or very low-dose heparin in combination with reduced doses of rPA, tPA, or streptokinase in 1188 patients. In the first arm of the trial, full-dose tPA was superior to reduced-dose streptokinase plus abciximab in achieving TIMI grade 3 flow at 90 minutes. However, reduced-dose tPA plus abciximab produced significantly greater normal flow rates at 60 minutes (72% vs 43%) and at 90 minutes (77% vs 62%) compared with full-dose tPA alone.

Rates of major hemorrhage were 6% with full-dose tPA, 7% with reduced-dose tPA plus abciximab and low-dose heparin, and 1% with reduced-dose tPA plus abciximab and very low-dose heparin.[46] Interestingly, the addition of abciximab to reduced dose tPA also improved microvascular perfusion. Improved microvascular perfusion was indicated by resolution of persistent ST-segment elevation in 59% of patients in the combination group versus 37% of those treated with tPA alone (Figure 3).[48]

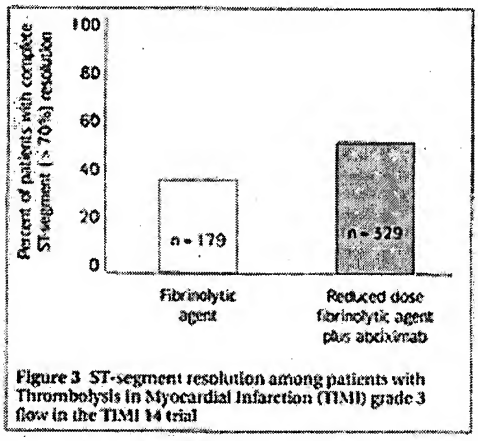

Figure 3 ST-segment resolution among patients with Thrombolysis in Myocardial Infarction (TIMI) grade 3 flow in the TIMI 14 trial

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The second phase of the TIMI 14 trial included patient groups that received full-dose rPA (10 U + 10 U, 30 minutes apart) alone and reduced dose rPA (5 U + 5 U) plus abciximab. Sixty- and 90-minute normal flow rates were achieved in 68% and 67% of the rPA-alone group and 70% and 69% of the combined rPA and abciximab group. Final TIMI flow results from the angiographic cohort are shown in the Table. There were no significant differences in major bleeding among the 4 treatment arms.[47]

In the SPEED (Strategies for Patency Enhancement in the Emergency Department) trial,[49] the pilot trial for GUSTO IV-AMI, 530 patients were given standard abciximab bolus plus infusion, full-dose rPA in a double bolus, or abciximab plus reduced-dose rPA at ascending single- or double-bolus doses. An important objective was to assess the effect of early administration of rPA and abciximab on outcomes of percutaneous coronary intervention.

The ongoing GUSTO IV trial is assessing the benefits of combination therapy in ST-elevation MI in approximately 17,000 patients in the Global Use of Strategies to Open Occluded Arteries IV-Acute Myocardial Infarction (GUSTO IV-AMI) study. Patients are to receive standard double-bolus rPA or half-dose double-bolus rPA plus abciximab, with the primary end point being 30-day mortality.

Facilitated Percutaneous Coronary Intervention

Significant delays in door-to-balloon time exist, even in the most advanced cardiac catheterization centers.[35,50] Based on the premise that outcomes may be improved by opening an infarcted artery with a fibrinolytic agent while a patient waits for catheterization, recent attention has turned toward a new paradigm of fibrinolysis prior to

coronary intervention. Studies of this combination performed in the early 1980s suggested that percutaneous coronary intervention following fibrinolytic therapy was associated with poorer clinical outcome and increased bleeding rates[51,52]; therefore, this practice was largely abandoned.

There have been several major developments since these early trials, including optimized use of heparin and aspirin, use of stenting, and improved angioplasty techniques. Major developments in pharmacology include the availability of new fibrinolytic therapy and GpIIb-IIIa inhibitors. The primary rationale for facilitated percutaneous coronary intervention is that early reperfusion will enhance the outcome of intervention. The feasibility of this approach was established by the Plasminogen Activator Angioplasty Compatibility Trial (PACT) study conducted by Ross and colleagues.[53]

Combination therapy in the Thrombolysis in Myocardial Infarction (TIMI) 14 trial: TIMI-grade flow results

	tPA (100 mg)	Reduced dose tPA (50 mg) + abciximab	rPA (10 U + 10 U)	Reduced dose rPA (5 U + 5 U) + abciximab
	n = 215	n = 148	n = 92	n = 100
TIMI-3 flow at 60 minutes (%)	43	71	68	70
TIMI-3 flow at 90 minutes (%)	62	74	67	69
Major hemorrhage (%)	6	4.6	2.9	6.5

rPA indicates recombinant plasminogen activator; TIMI-3 flow, thrombolysis in myocardial flow; tPA, recombinant tissue plasminogen activator.

Adapted with permission, from Coussement et al.[53]

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In this study, 606 patients were randomized to a bolus dose of tPA or placebo immediately prior to angiography. Patients were given a second bolus if TIMI grade 3 flow was present, or underwent percutaneous intervention if TIMI grade 0-2 flow was present. The primary end point was left ventricular ejection fraction on the predischarge ventriculogram. Patients receiving tPA had higher TIMI 2 (27.7% vs 19.5%) and TIMI 3 (32.8% vs 14.8%) flow rates than did placebo recipients. These findings thus suggested that early patency is associated with improvement of ventricular function, with tPA treatment being associated with achievement of early patency. No differences in bleeding events were observed between the treatment groups.

Support for the use of fibrinolytic therapy, particularly an rPA/abciximab combination, in patients undergoing percutaneous intervention comes from findings in patients for whom thrombolysis initially failed in the GUSTO III trial.[54] Comparison of outcomes in patients undergoing intervention 3.5 hours (median time) after full-dose thrombolysis showed that 30-day mortality was 3.6% for those who received abciximab compared with 9.7% for those who did not; although the rate of the composite end point of death, reinfarction, or stroke was similar in those receiving abciximab and in those who did not (12% vs 14%).

The effect of combination therapy on facilitating percutaneous coronary intervention has been evaluated in more detail in the SPEED trial.[49] In this trial, 323 patients underwent percutaneous coronary intervention after receiving a thrombolytic agent. It was found that those patients with TIMI grade 2 or 3 flow prior to intervention had a significantly greater procedural success rate than did those with TIMI grade 0 or 1 flow (93% vs 81%). Among patients receiving abciximab plus the rPA 5 U + 5 U double bolus, rPA alone, and abciximab alone, there were no significant differences in procedural success rates (88%, 85%, and 95%, respectively).

However, the rate of the composite clinical outcome of death, MI, or repeat revascularization after percutaneous coronary intervention was lower in the combination

treatment group (6.1%) than in the rPA-alone group (6.7%) or the abciximab-alone group (10.0%). These findings thus suggest that achieving coronary artery patency prior to intervention enhances outcome of intervention, even though there were no significant differences between treatments with regard to procedural outcome. As in the TIMI 14 combination trial, clinical outcomes were better with the rPA/abciximab combination. No increased bleeding risk was associated with use of combination therapy compared with that observed with use of rPA or abciximab alone.

Reteplase, because it can be administered as a double bolus, has the advantage of easier administration in the field. Indeed, a recent pilot study in 62 patients to assess the prehospital use of rPA indicated that fibrinolysis initiated in a prehospital setting is feasible and associated with a high rate of ST-segment resolution (approximately 80%) and favorable clinical outcome (49 of 55 patients with follow-up were alive at 30 days).[55] Prehospital thrombolysis with anistreplase was also shown to be very effective in the Grampian Region Early Anistreplase Trial (GREAT).[56] In this British study, treatment time was improved by more than 2 hours and 1-year mortality was halved (from 21.6% to 10.4%) when prehospital thrombolysis was performed.

Available data suggest that administering thrombolytic therapy prior to angioplasty improves clinical outcome. Facilitated percutaneous coronary intervention may yield advantages that include the ability to achieve earlier reperfusion in primary intervention patients, and avoidance of delay in use of lytic therapy while the decision of whether to perform percutaneous intervention is made. Other advantages include potential for maintenance of patency after successful lysis and improved patency when lysis is unsuccessful.

Nursing Considerations

Education Issues

Because the effectiveness of thrombolytic therapy is highly time dependent, rapid identification and treatment is an important focus of management of acute coronary syndromes. According to the National Heart Attack Alert Program, acute MI management is divided into 3 phases. Phase I consists of the patient component of treatment delay and phase II includes prehospital action (emergency medical services). Phase III is the hospital action component of treatment delay. Studies of treatment delay have shown that failure to seek care promptly (phase I) is the most common reason for treatment delay.[35]

Education efforts must be directed at the general public, with special emphasis on people with or at risk for coronary artery disease, and on patients with stable coronary artery disease. For education purposes and for protocol development, nurses must also be aware that the field of prehospital management of MI patients has expanded rapidly in recent years. As mentioned above, prehospital thrombolysis has been shown to improve outcomes in acute MI. Studies have also shown that prehospital electrocardiography improves outcomes.[57]

Conclusion

New therapies and approaches to treatment for acute coronary syndromes hold promise for improved clinical outcomes. Use of GpIIb-IIIa inhibitors against the platelet component of occlusive thrombi has been shown to produce significant benefits when used as an adjunctive therapy with percutaneous coronary intervention or alone as medical therapy in patients with unstable angina or non–Q-wave MI.

In the setting of acute MI, the combination of fibrinolytic agent and GpIIb-IIIa inhibitor treatment has been shown to result in more frequent and more rapid reperfusion, with some data indicating that there is improvement in myocardial function due to the effects of the antiplatelet agents in the microcirculation. The combination has been shown to be effective with reduced doses of the fibrinolytic component, which reduces hemorrhagic

risk associated with fibrinolytic treatment. Results of the GUSTO IV-AMI study will provide more information on the clinical benefits of this approach.

Facilitated percutaneous coronary intervention using fibrinolytic and antiplatelet therapy is emerging as a promising technique for achieving early reperfusion and shows potential for improvement in patency rates. This approach has been facilitated by the availability of fibrinolytic therapy that can be administered in bolus doses. Additional information on the clinical benefits of this approach will be provided by future studies.

Nurses must understand these rapidly evolving treatment options for unstable angina and acute MI because patients and other members of the healthcare team may call on them to provide guidance and answer questions. In addition, nurses with up-to-date knowledge are better able to play a role on hospital teams that develop treatment protocols, and to evaluate cost versus benefit issues related to these important drugs.

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Take The CE Test

Pharmacokinetics and Pharmacodynamics of AJW200, a Humanized Monoclonal Antibody to von Willebrand Factor, in Monkeys

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Abstract—The interaction between platelet glycoprotein Ib and von Willebrand factor (vWF) plays a crucial role in platelet-mediated thrombus formation under high-shear-stress conditions. The aim of this study was to investigate the antiplatelet profile of a humanized anti-vWF monoclonal antibody, AJW200. In vitro studies were performed with a modified cone-and-plate viscometer and human platelets. AJW200 inhibited high-shear-stress–induced platelet adhesion, aggregation, and thrombin generation, but it did not have such effects under low-shear-stress conditions. Although abciximab inhibited platelet aggregation under both shear stress conditions, it did not inhibit platelet adhesion and thrombin generation. In addition, the pharmacokinetics and pharmacodynamics of AJW200 were evaluated in cynomolgus monkeys. Sustained inhibition of ristocetin-induced platelet aggregation was observed over 24 hours, 6 days, and 2 weeks after a single bolus injection of 0.3, 1, and 3 mg/kg, respectively. Moderate prolongation of the bleeding time was observed at the doses of 1 and 3 mg/kg. Abciximab markedly prolonged the bleeding time at 0.4 mg/kg, at which concentration complete inhibition of ADP-induced platelet aggregation was observed. These results suggest that glycoprotein Ib–vWF blockade with AJW200 results in a sustained antiplatelet effect without extensive prolongation of the bleeding time, probably due to a shear-stress–dependent inhibitory action. (***Arterioscler Thromb Vasc Biol.* 2002;22:187-192.)**

Key Words: von Willebrand factor ■ platelets ■ shear stress ■ bleeding time ■ AJW200

Platelets play a crucial role in the pathophysiological progression of acute coronary syndromes (ACSs). Because the interaction of platelet glycoprotein (GP) IIb/IIIa with adhesive protein (ie, fibrinogen and von Willebrand factor [vWF]) is the final common pathway of platelet aggregation, various GPIIb/IIIa blockers have been developed. Abciximab is the Fab fragment of a chimeric monoclonal antibody (mAb) to platelet GPIIb/IIIa that has shown pronounced clinical benefit in the setting of percutaneous coronary interventions in patients with coronary heart deseases.[1–3]

Especially under high-shear-stress conditions, as observed in stenosed coronary arteries, for example, the interaction between platelet GPIb and vWF is involved in platelet-mediated thrombus formation.[4,5] Although some GPIb-vWF blockers have been reported to be effective against thrombus formation in various animal models,[6,7] none of the tested agents has yet been proved effective in clinical applications.

Previously, we reported that the murine IgG_1 against human vWF, AJvW-2, is a specific blocker of the GPIb-vWF interaction and inhibits arterial thrombus formation in various animal models.[8–11] These reports suggest that AJvW-2 may represent a new therapeutic agent for the treatment of patients with ACSs. However, "humanization" of AJvW-2 would be needed for clinical practice because murine IgG_1 is thought to have shown immunogenicity when administered to humans. Recently, we have succeeded in the humanization of AJvW-2 by grafting the mouse hypervariable regions onto a human IgG_4 framework, which is known to have minimal Fc functions.

The purpose of this study therefore was to characterize the in vitro antiplatelet profile of a humanized AJvW-2 (named AJW200) and to investigate the pharmacokinetics and pharmacodynamics of AJW200 after its bolus injection into cynomolgus monkeys.

Methods

Reagents

AJW200 (a humanized AJvW-2) is an IgG_4 humanized mAb to human vWF and derived from Sp2/0 mouse myeloma cells. Abciximab (c7E3 Fab, ReoPro™) was purchased from Eli Lilly Co.

Platelet Aggregation

Citrated blood was obtained from healthy volunteers by venipuncture, and platelet-rich and platelet-poor plasmas (PRP and PPP,

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respectively) were prepared by centrifugation. The platelet counts in PRP were adjusted to ≈250 000 per microliter by dilution with PPP. Platelet aggregation was measured with an aggregometer, the MCM Hematracer801 (MC Medical). After PRP was incubated with AJW200, abciximab, or vehicle at 37°C for 3 minutes, various agonists were added and platelet aggregation was monitored for 10 minutes as percent light transmission (with PPP set at 100%).

Shear-stress–induced platelet aggregation was measured with a modified cone-and-plate viscometer (Toray). Citrated PRP was incubated with AJW200, abciximab, or vehicle at room temperature for 10 minutes. The PRP sample was applied to the plate and exposed to shear stress at 25°C for 6 minutes. The cone was rotated at 1800 or 200 rpm, corresponding to shear stresses of 108 (high) or 12 (low) dyne/cm^2, respectively. Platelet aggregation was measured as percent light transmission.

Platelet Adhesion

Shear-stress–induced platelet adhesion was measured with a modified cone-and-plate viscometer (Toki Sangyo). Coverglasses were coated with human type III collagen (Sigma Chemical Co) and left undisturbed overnight at 5°C. Anticoagulated blood with D-phenylalanyl-L-prolyl-L-arginine chloromethylketone (Calbiochem; final concentration, 100 μmol/L) obtained from healthy volunteers was preincubated with AJW200, abciximab, or vehicle at room temperature for 10 minutes. After the coverglass was mounted to the plate of a modified viscometer, the blood sample was applied to the plate and exposed to shear stress at room temperature for 5 minutes. The cone was rotated at 250 or 60 rpm, corresponding to a shear rate of 1500 s^{-1} (high) or 360 s^{-1} (low). The coverglass was rinsed, fixed with methanol, and stained with May-Grunwald-Giemsa. Platelet adhesion was quantified by light microscopy, and the surface area coverage with platelets (as a percentage) was measured and calculated with the use of a computerized image graphic analyzer, Mac Scope. After analysis of 5 fields for each coverglass, the average value was calculated for each sample.

Platelet Procoagulant Activity

Platelet procoagulant activity was evaluated by measurement of thrombin amidolytic activity. Blood was obtained from healthy volunteers by venipuncture and anticoagulated with acid-citrate-dextrose (ACD; final concentration, 15%), and PRP was prepared by centrifugation. The platelet pellet was obtained by additional centrifugation of PRP and immediately suspended in ACD/HEPES buffer. A platelet suspension was centrifuged again, washed with ACD/HEPES buffer, and finally suspended in 1 mg/mL albumin/HEPES buffer. The platelet counts were adjusted to ≈200 000.

The platelet suspension was incubated with AJW200, abciximab, or vehicle at room temperature for 10 minutes. Immediately after 1 U/mL human vWF and 2 mmol/L $CaCl_2$ were added, the platelet suspension (400 μL) was applied to the plate and exposed to shear stress at 25°C for 3 minutes with a modified cone-and-plate viscometer (Toray). The cone was rotated at 1800 or 200 rpm, corresponding to respective shear stresses of 108 (high) or 12 dyne/cm^2 (low). The sheared platelet sample was incubated with 3.9 nmol/L factor Xa and 1.2 mmol/L $CaCl_2$ at 37°C for 1 minute and further incubated with 0.82 μmol/L prothrombin and 4 mmol/L $CaCl_2$ at 37°C for 1 minute. Finally, after the sample (25 μL) was incubated with chromogenic thrombin substrate S-2238 (225 μL) at 37°C for 1 minute, the reaction was stopped by adding 6% citric acid. The optical density was measured at 405 nm, and the amount of thrombin generated was calculated by use of a standard curve with α-thrombin (Sigma Chemical Co).

Ex Vivo Study in Monkeys

All procedures were performed in accordance with the institutional Animal Care and Use Committee of the Pharmaceutical Research Laboratories of Ajinomoto Co, Inc. Forty-five adult cynomolgus monkeys weighing 4.2 to 6.8 kg were divided into 9 treatment groups (n=5 each). AJW200 (0.03, 0.1, 0.3, 1, and 3 mg/kg body weight), abciximab (0.1, 0.2, and 0.4 mg/kg), or phosphate-buffered saline (control) was intravenously administered by bolus injection via the cephalic vein in a forearm. Blood collection and measurement of bleeding times were performed before and 5 minutes; 3 and 24 hours; 2, 4, and 6 days; and 2 weeks after drug administration. Unless platelet aggregation had recovered at 2 weeks, an additional measurement was performed at 3 or 4 weeks after administration. Citrated blood was obtained from the femoral vein and used for the measurement of platelet aggregation as well as hematological and coagulant parameters. Residual plasma samples were frozen and stored at −80°C.

PRP and PPP were prepared by centrifugation of citrated whole blood (4.5 mL) at room temperature at 700 rpm for 10 minutes and at 3200 rpm for 10 minutes, respectively. The platelet counts in PRP were measured with an automated cell counter (model E-4000, Sysmex) and adjusted to ≈300 000 platelets/μL by dilution with PPP. Platelet aggregation induced by ristocetin (final concentration, 2.5 mg/mL; Sigma Chemical Co) or ADP (final concentration, 20 μmol/L; Meiji Yakuhin) was measured by the change in light transmission for 8 minutes in an aggregometer (MCM Hematracer801, MC Medical). Ristocetin-induced platelet aggregation was performed in the control group and the AJW200-treated groups. On the contrary, ADP-induced platelet aggregation was performed in the abciximab-treated groups alone. Because frequent collection of large volumes of blood might have led to lethal anemia, both platelet aggregation assays were not performed for each animal. Plasma was obtained by centrifugation of the residual blood (0.5 mL) at 3000 rpm for 15 minutes, and prothrombin time and activated partial thromboplastin time were measured with an automated blood coagulation analyzer (Sysmex). Anticoagulated blood with EDTA-2K was obtained from the femoral vein, and hematological parameters were measured with an automated cell counter (Sysmex).

The template bleeding time was measured at the surface of the forearm with an automated spring-loaded device (Simplate R, Organon Teknika). Measurement was performed at serial intervals of 30 seconds up to a maximum time of 30 minutes, until the absorption of blood onto the filter paper had ceased, as determined by visual inspection.

Plasma vWF Antigen Level

A 96-well microtiter plate was coated with a rabbit anti-human vWF polyclonal antibody (10 μg/mL, Dako) and left at room temperature for 2 hours. The plates were blocked with 1% bovine serum albumin in phosphate-buffered saline at room temperature for 1 hour. Diluted plasma samples were added and incubated at room temperature for 2 hours. After being washed, the plates were incubated with a peroxidase-conjugated rabbit anti-human vWF polyclonal antibody (1:3000 dilution, Dako) at room temperature for 1 hour. After the plates were washed again, the numbers of bound vWF molecules were quantified by measuring the optical density at 490 nm. The plasma vWF antigen level was calculated as the percentage of the value of predosing plasma sample in each monkey.

Anti-AJW200 Antibody Formation

A 96-well microtiter plate was coated with AJW200 (5 μg/mL) at 4°C overnight. The plates were blocked with 1% bovine serum albumin/phosphate-buffered saline at room temperature for 2 hours. Diluted plasma samples were added and incubated at room temperature for 2 hours. After being washed, the plates were incubated with AJW200-absorbed, biotin-conjugated rabbit anti-monkey immunoglobulins (Nordic Immunological Laboratories) for 1 hour. After being washed again, the plates were incubated with a streptavidin–horseradish peroxidase conjugate for 1 hour. After a third washing step, bound monkey immunoglobulins were quantified by measuring the optical density at 490 nm. The plasma level of anti-AJW200 antibody was expressed as the optical density value. A positive reaction was defined as more than twice the predosing optical density value in each monkey.

Plasma Concentration of AJW200

A 96-well microtiter plate was coated with rabbit anti-human vWF polyclonal antibody (5 μg/mL, Dako) at room temperature for 2 hours. The plates were blocked with 1% bovine serum albumin/phosphate-buffered saline at room temperature for 1 hour. Diluted plasma samples and standard AJW200 solutions (0.0078 to 0.5

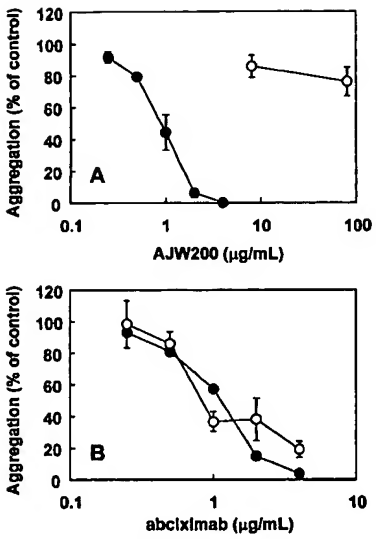

Figure 1. Line plots showing the effect of AJW200 (A) on shear stress–induced platelet aggregation compared with that of abciximab (B). Human PRP samples were incubated with AJW200, abciximab, or vehicle (control) for 10 minutes and exposed to high (●) or low (○) shear stresses at 25°C for 6 minutes. Data are represented as mean±SEM (n=4).

μg/mL) were added and incubated at room temperature for 2 hours. Simultaneously, a human vWF concentrate (ConfactF) was added to each sample at a final concentration of 0.025 U/mL (as a factor VIII:C). After being washed, the plates were incubated with peroxidase-conjugated mouse anti-human IgG_4 mAb (1:5000 dilution, Southern Biotechnology Associates, Inc) at room temperature for 1 hour. After a third washing step, bound antibody conjugate activity was quantified by measuring the optical density at 490 nm, and the plasma AJW200 level was calculated from a standard curve. The lower limit of quantification was 39 ng/mL. For pharmacokinetics modeling, a biexponential fitting of the plasma concentrations versus time was performed by weighted nonlinear regression analysis. Parameters of the 2-compartment model were then calculated by using a curve-fitting program.

Statistics

Data are presented as mean±SEM. In the in vitro study of platelet procoagulant activity, 1-factor ANOVA followed by Scheffe's and Dunnett's tests was used for statistical analysis of the comparison between the 3 control groups and of the efficacy of mAb compared with control high-shear-stress conditions, respectively. In the ex vivo study, repeated-measures ANOVA, followed by Dunnett's test, was used for statistical analysis of the efficacy of AJW200. Statistical analysis of the efficacy of abciximab was not performed. A value of $P<0.05$ was considered significant.

Results

Platelet Aggregation

AJW200 specifically inhibited human platelet aggregation induced by ristocetin and botrocetin, although abciximab inhibited any type of platelet aggregation (Table I; please see http://atvb.ahajournals.org). Also, AJW200 inhibited the high-shear-stress–induced platelet aggregation in a concentration-dependent manner, with an IC_{50} value of 1.0±0.1 μg/mL (Figure 1A). Low-shear-stress–induced platelet aggregation was not affected, even at a concentration of 80 μg/mL. On the contrary, abciximab inhibited both platelet aggregations at the same efficacy, and the IC_{50} value was 1.1±0.03 μg/mL under high-shear- and 1.2±0.5 μg/mL under low-shear-stress conditions, respectively (Figure 1B).

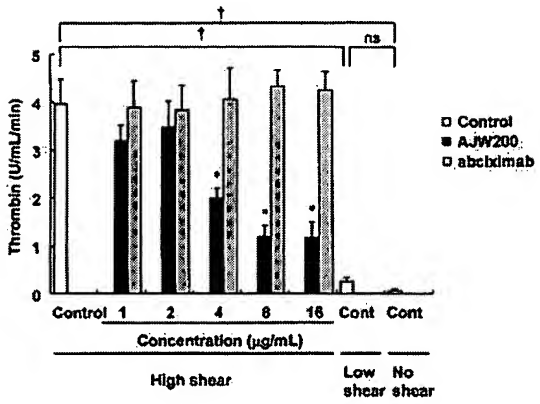

Figure 2. Bar graphs showing the effect of AJW200 on platelet procoagulant activity compared with that of abciximab. Washed human platelet suspensions were incubated with AJW200, abciximab, or vehicle (control) for 10 minutes and exposed to high, low, or no shear stresses at 25°C for 3 minutes after vWF and $CaCl_2$ were added. Data are represented as mean±SEM (n=4). *$P<0.05$ vs high-shear control by 1-factor ANOVA, followed by Dunnett's test. †$P<0.05$ by 1-factor ANOVA between control groups, followed by Scheffe's test.

Platelet Adhesion

Representative photomicrographs of platelet adhesion under shear stress conditions are shown in online Figure I (please see http://atvb.ahajournals.org). The surface coverage was 33.0±3.1% under high-shear- and 10.8±0.5% under low-shear-stress conditions. AJW200 specifically inhibited high-shear-stress–induced platelet adhesion, with an IC_{50} value of 2.6±0.2 μg/mL. No effect on low-shear-stress–induced platelet adhesion was observed, even at 64 μg/mL. In contrast, abciximab failed to inhibit platelet adhesion under both conditions, even at 20 μg/mL, although it abolished platelet aggregation.

Platelet Procoagulant Activity

The effects of AJW200 and abciximab on thrombin generation are shown in Figure 2. A significant increase in thrombin generation was observed under high shear stress compared with the low- or no-shear condition (4.0±0.5, 0.3±0.1, and 0.06±0.04 $U \cdot mL^{-1} \cdot min^{-1}$, respectively). AJW200 inhibited high-shear-stress–induced thrombin generation in a concentration-dependent manner, and significant inhibition was observed at 4 μg/mL. On the contrary, abciximab did not affect thrombin generation, even at 16 μg/mL.

Pharmacokinetics in Monkeys

AJW200 showed approximately dose-proportional pharmacokinetics over the dose range 0.03 to 3 mg/kg (Figure 3). Plasma AJW200 concentrations 5 minutes after intravenous administration of 0.03, 0.1, 0.3, 1, and 3 mg/kg were 0.55±0.06, 1.93±0.16, 7.07±0.45, 23.08±0.88, and 67.83±2.53 μg/mL, respectively. A biphasic decline in plasma concentration was observed for the 2 highest doses (1 and 3 mg/kg), although a possible slow-clearance phase could not be detected owing to the sensitivity limit of the assay for the 3 lowest doses (0.03, 0.1, and 0.3 mg/kg). The dominant terminal disposition phase was characterized by a half-life of 25.1±5.5, 20.6±2.5, 19.1±1.0, 43.3±3.4, and 63.0±5.8

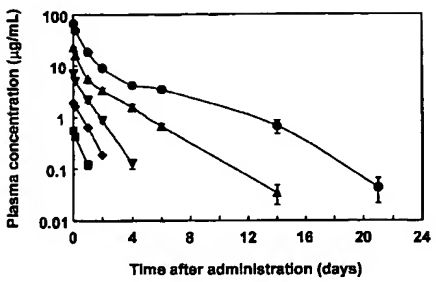

Figure 3. Line plots showing the pharmacokinetics of bolus doses of AJW200 in monkeys. AJW200 (■, 0.03; ◇, 0.1; ▼, 0.3; ▲, 1; and ●, 3 mg/kg) was intravenously administered by bolus injection at time 0. Data are represented as mean±SEM (n=5).

hours for 0.03, 0.1, 0.3, 1, and 3 mg/kg, respectively (Table II; please see http://atvb.ahajournals.org).

Pharmacodynamics in Monkeys

The antiplatelet effect of AJW200 and bleeding time prolongation are shown in Figure 4. AJW200 significantly inhibited the ex vivo ristocetin-induced platelet aggregation at 0.03, 0.3, 1, and 3 mg/kg. Complete inhibition of aggregation was observed at 0.3 mg/kg and above and lasted >24 hours at 0.3 mg/kg, for 6 days at 1 mg/kg, and for 2 weeks at 3 mg/kg. Inhibition of the ristocetin-induced platelet aggregation at 3 mg/kg AJW200 disappeared by 3 weeks for 4 animals and by 4 weeks for 1 animal. AJW200 did not affect bleeding times up to a dose of 0.3 mg/kg. Although AJW200 significantly prolonged the bleeding time at 1 and 3 mg/kg, a lengthy prolongation (≥30 minutes) was never observed, even at 3 mg/kg. No changes in hematological parameters (leukocytes, erythrocytes, hemoglobin, hematocrit, and platelets), coagulation parameters (prothrombin time and activated partial thromboplastin time), and plasma vWF antigen levels were observed in any of the groups (data not shown). In addition, antibody formation against AJW200 was not observed in any of the animals treated with AJW200 (data not shown).

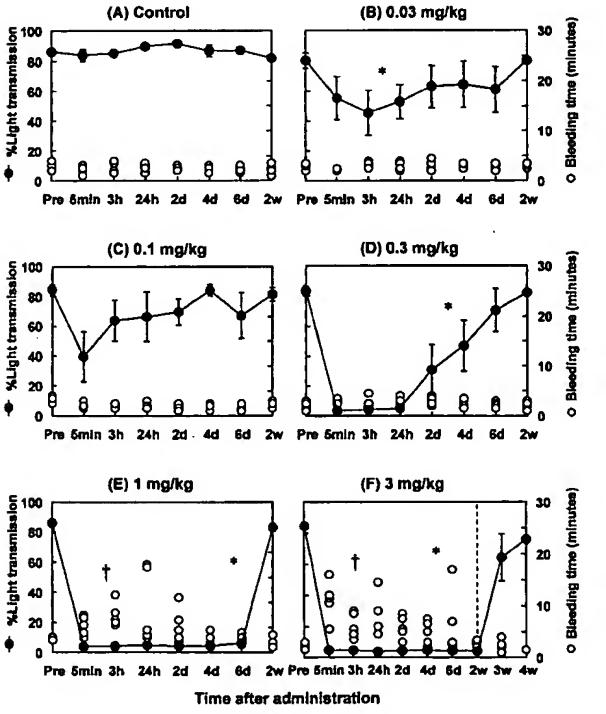

Figure 4. Graphs showing the effect of AJW200 on ex vivo ristocetin-induced platelet aggregation (line plot) and template bleeding time (dot plot) in monkeys (n=5). AJW200 was intravenously administered by bolus injection at time 0. Line plot data are represented as mean±SEM. *P<0.05 and †P<0.05 vs control group by repeated-measures ANOVA, followed by Dunnett's test, for the platelet aggregation studies and bleeding time measurements, respectively.

The relationship between the antiplatelet effect and bleeding time 5 minutes after AJW200 administration is shown in online Figure II (please see http://atvb.ahajournals.org). A wide window between the effective dose (on ristocetin-induced platelet aggregation) and bleeding time was observed in monkeys treated with AJW200. On the contrary, complete inhibition of ADP-induced platelet aggregation by 0.4 mg/kg abciximab was associated with a lengthy prolongation (≥30 minutes) of bleeding time. Inhibition of platelet aggregation rapidly disappeared (within 24 hours) after a bolus injection of abciximab (data not shown).

Discussion

The major findings of this study are that (1) a humanized mAb to vWF (AJW200) shows high-shear-stress–dependent inhibitory action against platelet activation and that (2) a bolus injection of AJW200 results in sustained inhibition of ristocetin-induced platelet aggregation without extensive prolongation of the bleeding time in monkeys. Previous clinical trials showed that abciximab significantly reduced ischemic complications in patients with ACSs.[1–3] However, it has also been reported that the high-shear-stress–induced platelet aggregation was augmented by the increase in plasma vWF levels in such patients.[12,13] Although these reports indicated that vWF-mediated platelet aggregation might be associated with the incidence of ACSs, the GPIb-vWF blocker is not yet available for clinical practice.

We have reported that AJvW-2, a murine mAb to human vWF, is a specific blocker of the GPIb-vWF interaction and is capable of preventing thrombus formation in vivo.[8–11] These studies suggest that AJvW-2 may be a new therapeutic agent for the treatment of patients with thrombotic disorders, including ACSs. To minimize the immunological responses against AJvW-2 when administered to humans, we humanized AJvW-2 by grafting the mouse hypervariable regions onto a human IgG_4 framework. In this study, AJW200 specifically inhibited human platelet aggregation induced by ristocetin as well as by botrocetin. In addition, under high-shear-stress conditions, the GPIb-vWF interaction plays a crucial role in platelet adhesion and aggregation.[4,5,14] AJW200 specifically inhibited high-shear-stress–induced platelet aggregation and adhesion; however, no effects were observed under low-shear-stress conditions.

Activated platelets can facilitate thrombin generation by providing a catalytic surface on which coagulation reactions occur and by releasing activated factor V, resulting in the formation of a secondary fibrin clot. A previous report indicated that tissue factor–induced thrombin generation in the presence of platelets was significantly inhibited by abciximab.[15] However, these findings were observed under static

conditions, and no study investigating such an effect of abciximab under shear-stress conditions was reported. The current study indicated that an ≈40-fold increase in thrombin generation was observed under high-shear-stress conditions compared with low- or no-shear conditions, indicating the significance of high shear stress for platelet activation. AJW200 inhibited high-shear-stress–induced thrombin generation, although no effect of abciximab was observed even at 16 μg/mL, at which concentration platelet aggregation was significantly inhibited. These in vitro findings suggest that abciximab therapy may result in the generation of many single platelets with a high procoagulant activity that are capable of adhering to collagen under high-shear-stress conditions, as occurs in stenotic coronary arteries, although platelet aggregation can be completely inhibited. Under these conditions, AJW200 inhibited platelet adhesion and the subsequent platelet aggregation, as well as activation due to inhibition of the GPIb-vWF interaction.

We have shown that AJvW-2 prevents arterial thrombosis in various animals.[8–11] However, the effective dose and especially the duration of efficacy cannot be extrapolated to humans, because murine mAb was an exogenous protein for these animals and therefore susceptible to rapid clearance. Also, whether administration of a humanized mAb to the GPIb-binding domain of vWF to nonhuman primates results in severe von Willebrand disease has not yet been investigated. Therefore, we investigated the pharmacokinetics and pharmacodynamics (especially the hematological functions) of AJW200 in cynomolgus monkeys. In this study, no antibody formation against AJW200 was observed in any monkey, probably owing to the lower immunogenicity of a humanized mAb. Also, no decrease in plasma vWF level was observed, probably because of the minimal Fc functions of IgG_4. Additionally, no changes in hematological and coagulation parameters were observed. These results indicate that the inhibitory effect of AJW200 on ristocetin-induced platelet aggregation might be due to specific inhibition of the GPIb-vWF interaction in monkeys.

Multimeric forms of vWF are composed of 250-kDa polypeptide subunits (monomers) linked together by disulfide bridges, and each monomer has 1 binding site for GPIb. If it is assumed that the plasma vWF level in cynomolgus monkeys is identical to that in humans (≈10 μg/mL [40 nmol/L as a monomer]) and that the stoichiometry of binding of the vWF monomer to AJW200 is 2:1 or 1:1, then the AJW200 concentration needed for saturated binding to plasma vWF can be calculated at 3 or 6 μg/mL. In this study, the mean plasma concentrations of AJW200 were 0.55, 1.93, 7.07, 23.08, and 67.83 μg/mL immediately after administration of 0.03, 0.1, 0.3, 1, and 3 mg/kg, respectively. These results suggest that at the 3 lowest doses tested, almost all of the AJW200 molecules in plasma may exist in the vWF-bound form immediately after administration and that AJW200 may be cleared at the rate to which it binds to vWF. Because excessive numbers of AJW200 molecules may also exist in the unbound form in the circulation at the 2 highest doses, it may persist for a time that is typical of IgG. In our preliminary experiment, binding of AJW200 to vWF in normal monkey plasma was saturated at ≈2.7 μg/mL (data not shown). Furthermore, saturated vWF occupancy was observed at the 3 highest doses in this study (data not shown). These results tend to support our hypothesis described above.

A bolus injection of 0.3 mg/kg AJW200 completely inhibited ristocetin-induced platelet aggregation, and significant inhibition was sustained for 24 hours without prolongation of the bleeding time. Longer durations of inhibition (6 days and 2 weeks) after administration of 1 and 3 mg/kg AJW200, respectively, were accompanied by a moderate prolongation of the bleeding time. In contrast, complete inhibition of ADP-induced platelet aggregation after a bolus injection of abciximab was accompanied by extensive prolongation (≥30 minutes) of the bleeding time, which rapidly disappeared within 24 hours. Because abciximab is routinely administered as a bolus injection followed by a 12-hour infusion in clinical practice, a high risk of bleeding tendency may last for 12 hours. Although many clinicians have indicated that prolongation of the bleeding time is not predictive of bleeding events in patients,[16] the low bleeding profile of AJW200 may be preferable in clinical practice. Furthermore, the plasma half-life of a humanized mAb is expected to be longer in humans than in cynomolgus monkeys, which is supported by a previous investigation that used a humanized mAb (IgG_4) to tumor necrosis factor-α.[17] A long-term antithrombotic effect could be achieved by a single bolus injection of AJW200 in future clinical practice. In addition, easy bolus administration would be preferable in an emergency situation.

In conclusion, the specific inhibition of GPIb-vWF interactions by a humanized mAb to vWF (AJW200) results in sustained inhibition of ristocetin-induced platelet aggregation without an extensive prolongation of bleeding time, probably owing to the high-shear-stress–dependent inhibitory action. AJW200 may become a drug of choice for the treatment of patients with ACSs.

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